Characterization of Gold Nanoparticles Functionalized with Thiolated Single-Stranded DNA

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Introduction:

Gold surfaces functionalized with deoxyribonucleic acid (DNA) are used in biomedical applications such as biosensing. When biosensors and other biomedical devices are placed in a biological environment, various reactions (analyte sensing, protein adsorption, etc.) will occur at their surface. For nanoparticles, where the surface-to-volume ratio is high, these surface properties are crucial and often unique from their larger scale counterparts [1].

Our work included functionalizing and characterizing gold nanoparticles (AuNPs) with thiolated single-stranded DNA (SH-ssDNA) in various buffers with different salt concentrations. We performed detailed surface analysis of the functionalized AuNPs using x-ray photoelectron spectroscopy (XPS). The SH-ssDNA functionalized AuNPs were then backfilled for varying amounts of time with hydroxyl-terminated alkylthiols to hinder DNA base-gold binding. Control flat Au surfaces were also functionalized with SH-ssDNA for comparison to the functionalized AuNPs.

Experimental Methods:

Materials. The 40-mer SH-ssDNA [5'(C5-Thiol) AGC TGC CCT GGT AGG TT TCT GGG AAG GGA CAG ATG ACA G 3'] was purchased from Trilink Biotechnologies (lot U17-0209-AC1A-A). Ultrapure water (resistivity >18.0 M Ω cm) used for preparing all aqueous solutions was purified by a Modulab Analytical research grade water system. Spectra/Por molecular porous membrane dialysis tubing was purchased from Sigma-Aldrich. Tubing-type 1 had a molecular weight cut off of 12-14,000 and tubing-type 2 had a molecular weight cut off 50,000 (wet in 0.1% sodium azide). All other chemicals were also purchased from Sigma-Aldrich.

Gold Nanoparticle Stability. UV/VIS measurements were performed to determine which buffer and salt conditions provided a stable environment for the AuNPs. A stable environment was considered one that did not cause the AuNPs to aggregate as identified by a change in color.

Thiol-DNA Assembly. Water, buffer, and SH-ssDNA were combined at the desired concentrations in a glass scintillation vial and mixed for 30 seconds. AuNPs were added, and when relevant, solution was mixed for 30 minutes before adding salt. The AuNPs were functionalized for 24 hrs before purification. Samples were dialyzed 2x in tubing-type 1 and 10x in tubing-type 2 (see materials) in 3500 mL water.

Backfilling Procedure. 97.55 μL of 9.0M hydroxyl-terminated alkythiol were added to 4 ml purified functionalized AuNPs. Samples were backfilled for varying amounts of time before being dialyzed 10x in tubing-type 2.

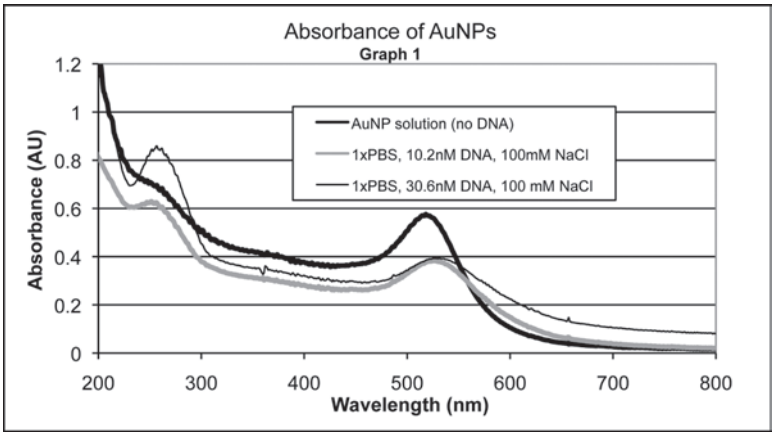
XPS Analysis. XPS measurements were performed on Surface Science Instrument S-Probe spectrometer with a monochromated Al-Kα x-ray source. Compositional survey, detailed scans (Au4f, C1s, O1s, P2p, S2p and N1s) and high resolution scans (C1s and N1s) were acquired. All measurements were taken at a 55° photoelectron takeoff angle. The computational data are averages of values from two samples, three spots per sample. Data analysis was performed on the Service Physics ESCA 2000 Graphics Viewer data reduction software.

Results and Discussion:

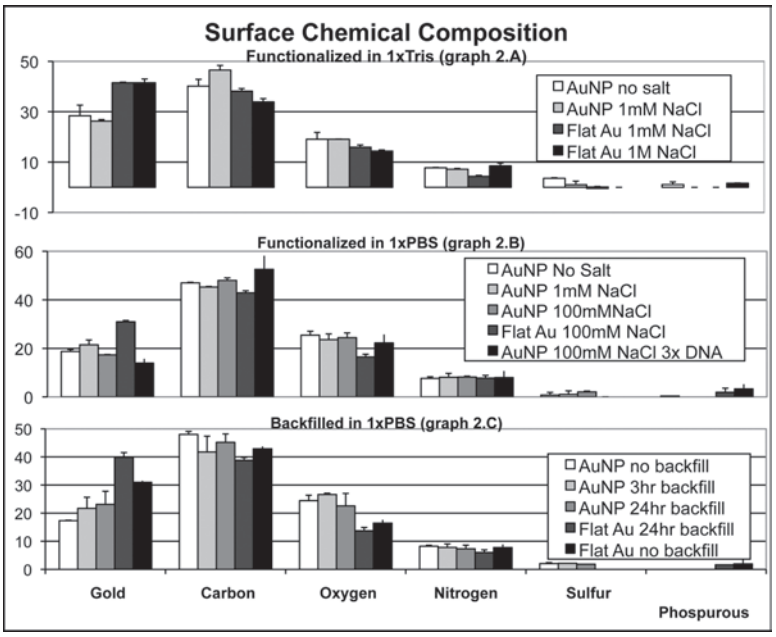
Stability of AuNPs. Adding SH-ssDNA to buffer before adding AuNPs helped decrease aggregation. Using this procedure, the highest stable salt concentrations in 1× Tris and 1× PBS were 1 mM NaCl and 100 mM NaCl, respectively. In both buffer systems, adding NaCl last allowed for a higher final salt concentration.

UV/VIS Absorbance of AuNPs (Graph 1). SH-ssDNA peaks appeared at 260 nm and gold peaks appeared around 525 nm. There was no SH-ssDNA peak in the AuNP solution before functionalization, as expected. The broadening of the gold peak in the functionalized samples indicated particle growth. This combined with the noticeable SH-ssDNA peaks suggested that SH-ssDNA had been attached to the AuNPs.

Surface Chemical Composition of AuNPs (Graph 2). Increasing the salt concentration of a sample was previously shown to increase the SH-ssDNA loading onto the surface by



Graph 1



Graph 2

shielding the DNA-DNA repulsion forces [2]. However, SH-ssDNA loading on the AuNPs was not significantly affected by the salt concentration. An increase in loading would result in a lower percentage of gold seen on the surface [3]. This was not the case in Tris buffer (Graph 2.A) or PBS (Graph 2.B).

It is possible the SH-ssDNA concentration was too low to be affected by the repulsion forces. However, increasing the SH-ssDNA concentration by 3x (Graph 2.B) did not significantly change surface composition. In PBS, AuNPS exhibited similar nitrogen composition compared to flat surface. In Tris, there were similar phosphorus compositions. Some samples also had high amounts of sulfur. This was most likely due to minor contamination.

Graph 2.C shows the chemical surface composition of functionalized AuNPs backfilled with 6-mercapto-1-hexanol for 0, 3, and 24 hrs. The time allowed to backfill had little effect on alkythiol coverage on AuNPs. That is, the 3 hrs time range was long enough for the backfilling to be equilibrated; therefore, shorter times (< 3 hrs) are recommended for future studies. The flat surface comparison is also shown on Graph 2.C. Both the AuNPs and flat gold exhibited an increase in percentage of gold on the surface as backfilling occurred. This seems counter intuitive, but the hydroxyl-terminated alkylthiols being added were significantly shorter than the 40mer SH-ssDNA. Hence, the alkylthiols attenuated the gold signal less than the SH-ssDNA [3].

Conclusions:

To develop understanding of the structure-function relationship for biomedical devices and optimize their performance, their surface properties must be characterized. In this study, 14 nm diameter AuNPs were functionalized with SH-ssDNA in Tris and PBS buffer in varying salt concentrations. Using surface analysis techniques for characterization, we determined adding salt did not enhance SH-ssDNA assembly for the DNA concentration used in this study. Control flat gold surfaces were subjected to similar conditions. All AuNP samples had lower percentages of gold than the flat surface samples. The curved surface of AuNP may have enabled higher SH-ssDNA loading. Also, compared to flat surfaces, the XPS organic overlayer signals are enhanced relative the underlying gold signals for AuNPs [4].

Further characterization and optimization of the DNA coated AuNPs is currently underway.

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