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Shape-Tunable Plasmonic Gold Nanosensors for Quantitative Circulating Tumor DNA Screening

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Abstract

Cancer has been the leading cause of death worldwide with 1.8 million new cancer cases and over 600,000 deaths in 2020 alone. As the focus of cancer diagnosis has become shifted towards personalized care, molecular diagnostics is a promising method for individualized monitoring of patient tumors, as well as its genetic and phenotypic characteristics. In recent years, liquid biopsy has become a popular method of minimally invasive cancer detection. circulating tumor DNA(ctDNA) is a particularly promising biomarker due to its capability to capture both the genetic and epigenetic changes within a tumor. Moreover, its short, two hour, half-life allows for a representative snapshot of the tumor's genetic profile. Enrichment and quantitation of ctDNA, however, is a challenge because of its low concentration and the presence of non-tumor specific cell-free DNA in the blood.

This study involved designing and fabricating gold nanospheres, nanorods, and nanobipyramids using chemical synthesis to examine the effects of nanoparticle geometry on plasmonic sensitivity. From plasmonic theory, we hypothesized that particles with sharp angular geometries have higher resonance sensitivities due to plasmonic hotspots. We developed electromagnetic simulations to theoretically analyze the correlation between angular geometry and plasmonic resonance and sensitivity. Nanosensors were synthesized through chemical functionalization of the gold nanoparticles, which were conjugated with Peptide Nucleic Acid (PNA) probes for rapid detection of ctDNA. We used a sequentially complementary probe design to capture the G12D variant in Exon 2 of the *KRAS* gene, a variant extremely relevant to pancreatic and colorectal cancer. The nanosensors were dispersed on-chip using microfluidics printing, which allowed for rapid sample delivery. We measured the location of the plasmonic resonance peak on the three nanogeometries at five concentrations (0 ng/mL, 25 ng/mL, 50

ng/mL, 75 ng/mL, 100 ng/mL) of ctDNA-spiked healthy patient serum samples.We found limits of detection at 0.6 nanograms/mL for the nanobipyramids and 1.2 nanograms/mL for the nanorods, approaching the clinically relevant range, with the nanobipyramids being two times as sensitive as the nanorods. This was among the first studies comparing plasmonic geometries at nanoscale for quantification of tumor-derived mutations in a clinically relevant scenario. The developed platform may open new opportunities for a wide range of applications from cancer management to drug discovery at molecular scale towards precision healthcare.

Introduction

Liquid biopsy is the detection of biomarkers that shed off of the tumor into peripheral fluid samples, rather than the collection of a tissue sample at the tumor site [1], [2]. This could allow for better patient screening, diagnosis, and monitoring through insight into the molecular fluctuations within the tumor in real time [3]. One biomarker of interest, circulating tumor DNA (ctDNA), provides insight into tumor mutational status, but often requires lengthy clinical workflows such as polymerase chain reaction (PCR) to quantify [4], [5]. There are few platforms for rapid, sample-to-answer detection of circulating tumor DNA due to the need for nucleic acid amplification.

Plasmonic nanosensors employ the highly sensitive electric fields at the surface of a metal and dielectric [6], [7]. These fields are sensitive to binding events at this interface, and can be used to elucidate the presence of biomarkers of interest through biorecognition at the surface of nanoparticles [8], [9]. Shape effects of plasmonic particles have been studied extensively from a theoretical and electromagnetic standpoint, with a number of papers describing the effects of particle geometry on the electric field at the surface of the particle [10], [11]. Our prior work

used commercially available gold nanorods for sequence-specific plasmonic detection of circulating tumor DNA [8], [9]. In this work we extend those principles to novel nanoparticle geometries to improve the sensitivity and limit of detection of plasmonic nanosensors for ctDNA.

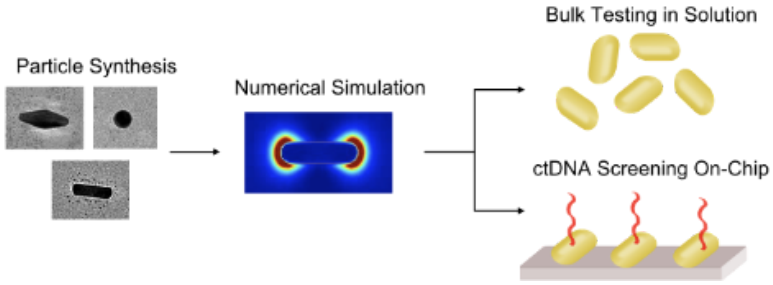


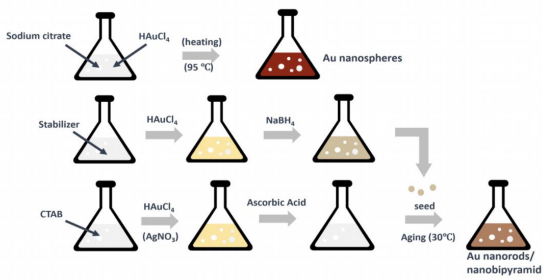
Figure 1: Study Workflow from particle synthesis to simulation to testing both in solution and on-chip.

Few papers have carefully rationally designed nanoparticles for specific biosensing applications and actually demonstrated this approach from design to biomarker capture [12]. In this paper, we design three geometries of nanoparticles for ctDNA capture. We show a method for extremely uniform nanoparticle synthesis and develop electromagnetic simulations to model each nanoparticle shape. We then test the nanoparticles both in solution and on-chip to determine plasmonic sensitivity as compared to the models. We performed this workflow for 3 shapes of nanoparticles to determine our ability to rationally design nanoparticles for improved plasmonic ctDNA screening. The workflow for this study can be found in Figure 1.

Materials and Methods

A. Nanoparticle Synthesis and Characterization

Gold nanorods, nanospheres, and nanobipyramids were synthesized in solution. Gold nanospheres were synthesized through stirring and heating chloroauric acid solution in sodium citrate with the molar ratio of 1:10. The gold nanorods and nanobipyramids were fabricated using a seed-mediated method. Throughout both synthesis processes, CTAB was the surfactant to prevent coagulation and serve as a template. The gold seed solution was mixed with a growth solution and left overnight. All nanoparticles were characterized using transmission electron microscopy.



Multiple Au NPs	Stabilizer in Au seeds	$AgNO_3$
Nanospheres	N/A	No
Nanorods	CTAB	Yes
Nano-bipyramids	Sodium Citrate	Yes

Figure 2: Schematic of Nanoparticle Synthesis and conditions for fabrication of nanospheres, nanorods, and nanobipyramids.

B. Development of Electromagnetic Simulation

The resonance features of the synthesized particles were simulated using CST Microwave Studio. The synthesized nanosensors were created in the simulation studio and were simulated as the center of a cube in the chosen refractive index. These spectra were run in both water and in media that represented water glycerol mixtures. The dimensions of the cube were optimized to minimize coupling and boundary effects. We were able to simulate both the shape of the spectrum as well as the sensitivity to changes in bulk refractive index.

C. Bulk Refractive Index Testing

To mimic the nanoparticle surface refractive index changes in ctDNA binding, we characterized the particles in water-glycerol mixtures of differing concentrations. After the gold nanoparticles were centrifuged at 6000rpm for 8 minutes, and the supernatant was removed. The collected nanoparticle pellets were incubated in water-glycerol mixtures with varying concentrations(0%, 20%, 50%, 80%, 100%) to examine the response of the surface plasmon peaks to refractive indices (RI). The complete absorbance spectrum was taken from each nanoparticle sample to observe the red shift in the longitudinal peak values due to the changing concentration and refractive index of the mixture. The Tecan Spark 10M Microplate reader from 400nm to 1000nm with a step size of 1nm was used to capture the spectrum.

D. Nanoparticle Microfluidic Printing on Chip

The synthesized nanosensors(nanorods, nanospheres, and nanobipyramids) were dispersed on-chip using microfluidic printing. The solution of deionized (DI) water and gold nanoparticles were dispersed into a 96-well plate at a concentration of OD 0.25 (absorbance at peak wavelength). Bare glass slides were functionalized for ten minutes in anhydrous ethanol before thorough drying and microfluidic printing. Then, the nanoparticles were printed using the Carterra Continuous Flow Microspotter onto a glass slide. Optical imaging was used to visualize printed nanoparticle areas.

E. Nanoparticle Conjugation and circulating tumor DNA Testing

Gold nanoparticles were washed thoroughly before dispersal onto an activated glass slide.The gold nanoparticles dispersed on chip were conjugated with peptide nucleic acid (PNA)

probes complementary to the G12D variant in Exon 2 of the KRAS gene.The nanoparticles were incubated with 2.5mg/mL of DSP (dithiobis succinimidyl propionate) in DMSO for 30 minutes, then coupled to 1mg/mL of the peptide nucleic acid probe(PNABio) in TE buffer for one hour. The sensor was then washed and ready for testing. A FERGIE Integrated Spectrograph (Princeton Instruments) coupled to an optical microscope were used for capturing the spectra. The spectra were then analyzed and processed in MATLAB, which calculated the extinction spectrum and peak location. Peak wavelength location was calculated from the center of mass of peak boundaries.

Results and Discussion

A. Seed-mediated Nanoparticle Synthesis & Characterization

The synthesized nanoparticles were extremely uniform in size, with the gold sphere having a 20nm radius, the gold nanorods measuring 13nm in diameter by 45nm in length, and the nanobipyramids measuring 25nm in width and 70nm in length. The exceptional uniformity of the nanoparticles allows the resonance peaks of the particles to be narrow, with a low full width at half maximum, allowing for enhanced sensing capabilities. Using ImageJ and MATLAB, we quantified the length and widths of the particles and also determined a relatively narrow standard deviation of measurements, confirming that the synthesized particles are uniform.

B. Theoretical & Experimental Analysis of Resonance

We compared the experimentally determined absorbance spectra of each of these particles to the theoretically determined extinction spectra from simulation. After baseline

correction of peak location for the nanorods, we could find extreme similarity in the resonance features for all three of the particles. The nanospheres, as expected, had only one plasmonic peak while the nanorods and nanobipyramids had two plasmon peaks for each of the characteristic dimensions. These data can be seen in Figure 3**,** where the simulated results are represented by dashed lines and the experimental results are represented by solid lines. Of note is the fact that the nanobipyramid peak is narrower than the nanosphere or nanorod peak, indicating that it could be potentially advantageous to sense small changes in peak location. This agreement on resonance features shows that our simulations are able to accurately predict the plasmonic response of each particle geometry in solution.

Table 1: Bulk Refractive Index Sensitivity in Simulation and Experiment for each Nanoparticle Geometry.

Shape	**Refractive Index Sensitivity (nm/RIU)**	
	Simulation	***Experimental***
Gold Nanosphere	73	122
Gold Nanorod	212	228
Gold Nanobipyramid	367	302

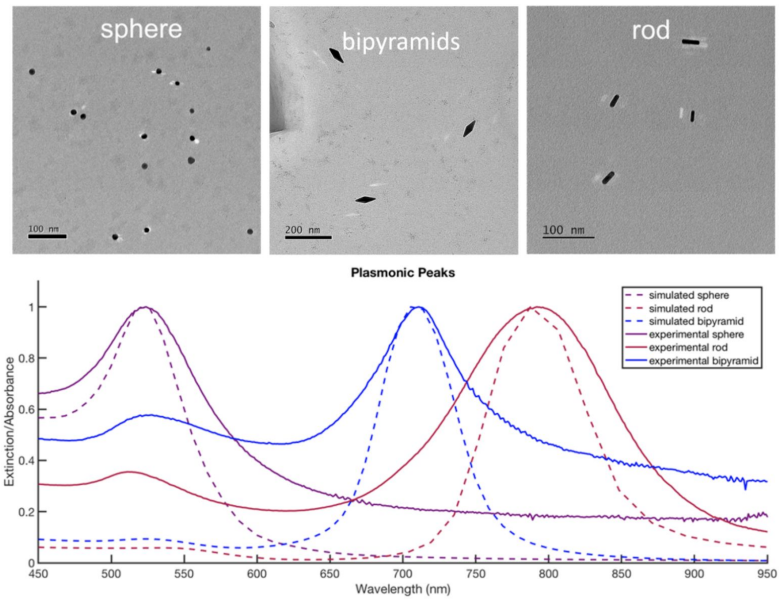


Figure 3**:** Electron Microscopy Images of Synthesized Particles. (bottom) Comparison of baseline (resonance feature wavelength location) corrected plasmonic extinction peaks from simulation and absorbance peaks from experiment..

C. Bulk Refractive Index Sensitivity

In addition to significant agreement in plasmonic spectra shape and resonance features, we also found qualitative agreement in bulk refractive index sensitivity. We tested the simulated particles in three different refractive index mixtures representing suspensions of water and glycerol and calculated the locations of the peaks as a function of refractive index – the slope of this line is the refractive index sensitivity. We did the same analysis experimentally with five mixtures of glycerol in water. The results of these experiments and simulations can be found in Table 1**.** This qualitative comparison shows significant agreement between simulation and experiment, demonstrating that the nanospheres are the least sensitive to changes in refractive index and that nanobipyramids are the most sensitive. These results agree with the theory as well, as high aspect ratio geometries with sharp corners are known to present more sensitive plasmonic resonances [17].

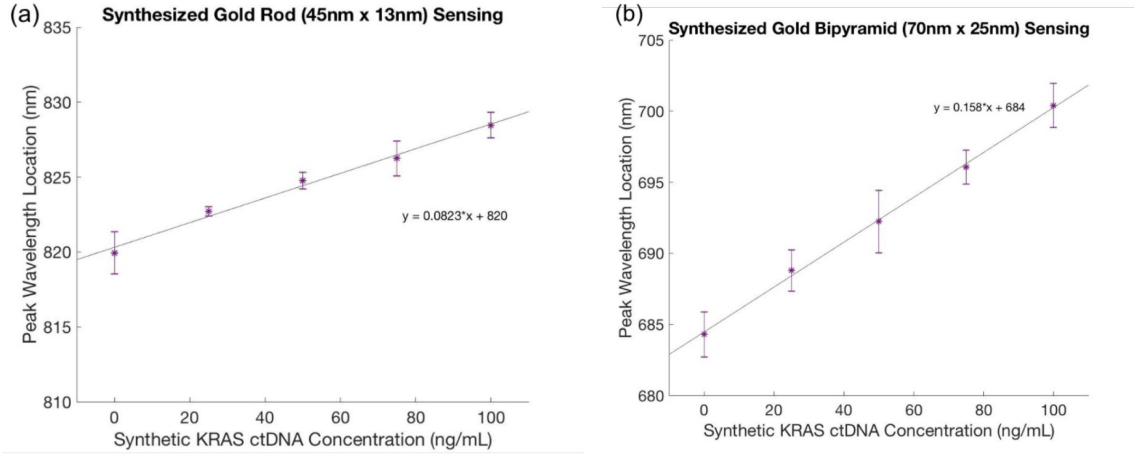


Figure 4: Synthetic ctDNA screening using synthesized gold nanorods and gold nanobipyramids. Gold nanospheres are not pictured due to resonance location of plasmonic peaks.

D. Circulating Tumor DNA Screening

We then took this study one step further and tested the gold nanorods and gold nanobipyramids for sequence-specific synthetic ctDNA screening. We chose the G12D sequence of the *KRAS* gene because mutations in this location are common in patients with pancreatic ductal adenocarcinoma, a disease that is notoriously challenging to diagnose [18], [19]. We conjugated and tested these particles dispersed on-chip with 0ng/mL, 25ng/mL, 50ng/mL, 75ng/mL, and 100ng/mL of target synthetic ctDNA, which approach the clinically relevant concentration range We found that the gold nanobipyramids were twice as sensitive as the gold nanorods to increases in synthetic ctDNA, which was also predicted by their bulk refractive index sensitivities.

Conclusion

In conclusion, we have demonstrated a versatile methodology for the rational design of plasmonic gold nanoparticles for nucleic acid screening. From initial particle design conception through to electromagnetic simulation and testing, our framework allowed for the development of nanoparticle geometries with enhanced sensitivity.

We found exceptional agreement between our electromagnetic simulations and our experimental results, indicating that numerical modeling can allow us to predict the resonance spectra of any particle geometry we design. We also found that these simulations can adequately predict bulk refractive index sensitivity, as we found good qualitative agreement between our simulated and experimentally determined measurements.

Even more interestingly, our theoretical determination that the nanobipyramids would be the most sensitive of the nanoparticle geometries carried through to the sensing of synthetic ctDNA. This is to our knowledge one of the first reports of careful nanoparticle design from simulation to refractive index testing to clinically relevant biomolecule sensing. This workflow shows immense promise for the design of nanoparticles for sequence-specific detection of ctDNA and other clinically relevant biomolecules. This approach could eventually be used in a range of patient samples, including blood.

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