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Plasmonic Nanoparticle Dimers for Optical Sensing of DNA in Complex Media

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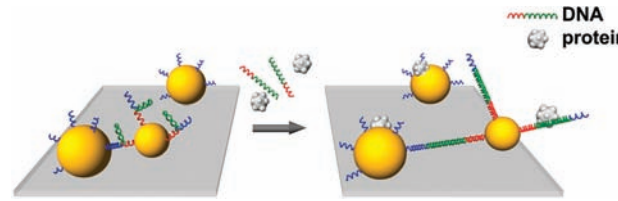
Abstract: We introduce a new sensing modality based on the actuation of discrete gold nanoparticle dimers. Binding of the target DNA leads to a geometrical extension of the dimer, thereby yielding a spectral *blue shift* in the hybridized plasmon mode as detected by single nanostructure scattering spectroscopy. The magnitude and opposite direction of this shift enabled us to spectroscopically distinguish the target from nonspecific binding and to detect the target in complex media like serum.

An ideal sensor has high sensitivity, specificity, and versatility.[1] For biosensors, both "label-free" sensing[2] and the ability to detect an analyte in complex media with minimal interference from nonspecific binding are highly sought goals.[3] Optical detection via surface plasmon resonance (SPR)[4] or localized surface plasmon resonance (LSPR)[5] is one of the most widely studied label-free sensing modalities. Methods based on the wavelength shifts of LSPR extinction or scattering upon analyte binding are particularly attractive due to their sensitivity and ease of integration into chip-based devices.[6,7] However, in SPR and LSPR sensors, it can be difficult to distinguish between binding of the target and nonspecific adsorption because both processes lead to an increase in the local refractive index. Selectivity for SPR and LSPR sensors is thus usually achieved via careful control of surface chemistry to obtain nonfouling properties.[8] On the contrary, little if any work has explored the possibility of tailoring LSPR-based sensors to exhibit qualitatively different spectral responses to specific and nonspecific binding. To address this challenge, we introduce the concept of using supported, actuatable nanoparticle structures as LSPR sensors. Here the detector molecule used to link the nanoparticles must be designed to undergo a structural change upon target binding. For proof of concept, we demonstrate DNA sensing. We show that these actuatable nanoparticles maintain the traditional advantages of LSPR sensors,[6] including low detection limit and the potential for single-nanostructure sensing, while at the same time providing for a qualitatively different spectral response to specific and nonspecific binding, thus allowing for simple spectroscopic detection of a target in complex media.

We chose to explore closely spaced supported nanoparticle dimers to achieve the blue shift in spectral response that we desire for target binding. In these nanostructures, the energy of the hybridized plasmon mode depends on the separation distance between the individual nanoparticles, where an increase in separation distance raises the energy of the bonding plasmon.[9] This dynamic response has recently been demonstrated.[10−12] While there are a few reports of plasmonic sensors with analyte detection by spectral blue shift, they rely on the disassembly of large aggregates in solution.[13,14] Herein we show that sensing of DNA via actuation of the interparticle separation in a supported nanoparticle dimer, as monitored discretely by single-particle spectroscopy, can serve as a viable platform for sensing.

Scheme 1 depicts our approach. We fabricated gold nanoparticle (AuNP) dimers (particle diameters of 100 and 60 nm) with one particle of the dimer anchored to the substrate via aminosilane chemistry. We identified dimers from single NPs and larger aggregates via both polarized spectroscopy and correlated SEM. Both particles are heavily loaded with oligonucleotides, and the two are linked together through a hairpin loop (see Supporting Information). In the initial state, the particles are close together. When the target, a complementary (cDNA) strand to the hairpin sequence, is introduced, the hairpin loop unzips leading to a geometrical extension of the DNA and an increase in the interparticle separation.[11] This increase in turn leads to a blue shift in the dimer scattering resonance.

Scheme 1. Geometric Extension Motif for Biosensing[a]



[a] Note that the protein depicts the nonspecific binding.

Figure 1a shows the single-particle dark-field scattering spectra from a typical hairpin-linked dimer in phosphate buffer solution for both polarized and unpolarized light. Before addition of the target, the unpolarized dimer spectrum consists of a clear peak at 635 nm with a shoulder on the blue edge close to 560 nm. Since the nature of plasmon coupling between individual nanoparticles in the dimer is different depending on the orientation of the excitation field, there exist two separate plasmon resonances. One, a red-shifted peak at 634 nm under parallel polarization, we assign to the bonding dipole plasmon.[9] The second peak, detected under perpendicular polarization, is a weakly coupled transverse mode at 585 nm.[9] After addition of the target strand, the bonding dipole plasmon blue shifts to 622 nm while the transverse resonance remains at 586 nm. Actuation of the separation distance leads to a blue shift only in the bonding dipole plasmon peak, also detectable in the unpolarized spectrum. The data we present below are from light polarized parallel to the dimer axis.

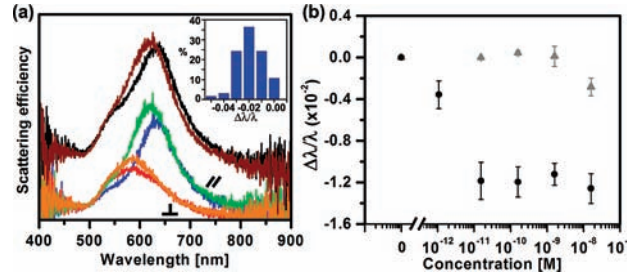


Figure 1. (a) Scattering spectra of a hairpin-linked dimer before and after exposure to target DNA. The spectra in black, blue, and red are before the addition of DNA while those in brown, green, and orange are after the addition, for light unpolarized, parallel polarized, and perpendicular polarized, respectively, to the dimer axis. The inset shows the histogram on the distribution of the fractional shift upon hairpin extension for a sample size of 66 dimers. (b) Plot of fractional shift vs different concentrations of target DNA for dimers (black circle) and single NP (gray triangle).

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To explore the viability of this sensing modality, we collected scattering spectra of dozens of DNA-actuated dimer pairs. The average spectral peak for 66 dimers shifted from 626 ± 2 nm to 615 ± 2 nm upon binding of the target DNA with no shift observed upon addition of noncDNA controls. The fractional shift, defined as $\Delta\lambda/\lambda$, is -0.019 ± 0.001, and the distribution of shifts is shown in the inset. Using the plasmon ruler calibration reported for dsDNA on symmetric dimers of 87 nm in particle diameter,[15] this spectral shift translates to an increase in average interparticle distance from 14 ± 1 nm to 23 ± 2 nm upon extension of the hairpin, in close agreement with the expected sequence lengths, but differing slightly from the distances reported previously.[11,16] However, as those authors pointed out, the length per base pair may be influenced by particle size and the number of interparticle links.

Next, we evaluated the sensitivity of detection by the linked dimers. Figure 1b shows $\Delta\lambda/\lambda$ as a function of target DNA concentration for both dimers and single particles (for reference). A significant decrease in $\Delta\lambda/\lambda$ can be detected as low as 10^{-11} M with dimers, suggesting that the detection limit is ~10 pM, or ~0.1 μg/L, which is competitive with many nonamplified label-free DNA sensing methods.[3] Furthermore, we estimate that the number of DNA strands linking a dimer is ~10 to 100 based on a surface coverage of 18 pmol/cm^2 and junction area of ~100−1000 nm^2.[17] Hence it may be possible to approach the single-molecule detection limit by asymmetrically functionalizing the NPs to localize a single hairpin in the junction area linking the two particles.[18]

We demonstrate that the spectral blue shift of the dimers allows us to distinguish target binding from the nonspecific binding of interfering macromolecules. Figure 2a shows $\Delta\lambda/\lambda$ as a function of time for both single NPs and dimers exposed to target DNA or noncDNA controls (1 μM) in the presence of bovine serum albumin (BSA, 30 mg/mL). At these BSA levels, both the single nanoparticles and the control dimers exposed to noncDNA show an overall red shift ($\Delta\lambda/\lambda$ of +0.004 and +0.008, respectively) due to the nonspecific binding of the BSA and concomitant increase in the local refractive index (RI = 1.5−1.6 for BSA[19] vs RI = 1.33 for aqueous solution). In contrast, dimers exposed to target DNA in the presence of BSA exhibit a significant blue shift indicative of hairpin extension despite the interfering BSA (albeit at a slower rate (τ = 2.9 h) than that in the absence of BSA (τ = 10−20 min)). With the target, the dimers exhibit a final average $\Delta\lambda/\lambda$ of -0.0012 ± 0.002 (both opposite in sign and larger in magnitude than those of the control dimers), thus validating our hypothesis that dimer extension can be used to differentiate specific and nonspecific binding.

Finally, we tested the performance of the nanoparticle dimers in a complex medium, fetal bovine serum. Figure 2b shows that $\Delta\lambda/\lambda$ increases monotonically with increasing concentration of serum in the presence of target DNA. We achieved target detection, as indicated by a negative $\Delta\lambda/\lambda$, in a serum concentration as high as 50%. At 75% serum, the competitive nonspecific binding of serum proteins overwhelms the hairpin extension process and a spectral red shift $\Delta\lambda/\lambda$ of $+0.007 \pm 0.002$ was observed instead. Note that the exact concentration of serum that can be tolerated will depend on the concentration of the target, the interaction between the target and constituents of the serum, and the nature of the interference. Additional experiments show that the dimers failed to extend if they were first incubated with 50 or 75% of serum followed by target DNA. Nevertheless, while nonspecific binding at high protein levels can prevent detection, the qualitative difference in spectral shifts enables one to determine the nature of the binding, thus preventing a false positive.

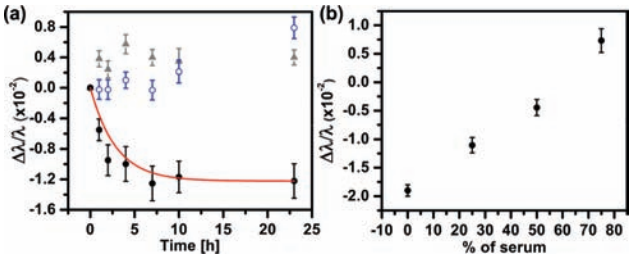

Figure 2. (a) Fractional shift as a function of time for dimer (black circle) and single particle (gray triangle) exposed to target DNA in 30 mg/mL BSA and for dimer exposed to noncDNA in BSA (open blue circle). The exponential decay fit is $y = 0.0122 \exp(-x/2.88) - 0.0122$. (b) Fractional shift of dimer for different concentrations of serum solution containing target DNA.

In summary, we have shown that the target-induced actuation of nanoparticle dimers can be an effective mechanism for optical biosensing in complex media at the single nanostructure level. With proper linker functionalization, the method could enable sensing of single molecular binding events using LSPR shifts. We envision that this mechanism could be applied to the detection of ions and small molecules using appropriately designed biological linkers that would undergo geometrical extension upon analyte binding, thus adding spectral response as a means to improve the specificity of LSPR- and SPR-based sensing.

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Supporting Information Available: Experimental details, optical and SEM images, additional scattering spectra, and sensing data. This material is available free of charge via the Internet at http://pubs.acs.org.

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