THE DISASSEMBLY OF A CORE-SATELLITE NANOASSEMBLED SUBSTRATE FOR COLORIMETRIC BIOMOLECULAR DETECTION

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ABSTRACT

We present the fabrication of gold nanoassemblies constructed on a two-dimensional substrate, demonstrate colorimetric biomolecular sensing functionality via protease-mediate disassembly, and systematically analyze the “naked eye” detection properties by calculating a figure of merit. Optimization of the scattering spectra of the core-satellite nanoassemblies maximizes the signal-to-noise ratio during biosensing and enables the large colorimetric shift of this assay from orange (>630 nm) to green (~560 nm) via the observation of scattered light in dark field (DF). Nanoconstructs with 50 nm cores and 30 nm or 50 nm satellites were found to be ideal for colorimetric readout.

KEYWORDS: Nanoparticle, Colorimetric, Biosensor, Plasmonic, Nanoassembly

INTRODUCTION

Previous reports of disassembling colorimetric nanomaterials on a substrate have included the resuspension of nanoparticles released from nanolattices dried on hydrophobic paper or constructed on a fluid lipid bilayer membrane [1,2]. Unlike previous biosensors, we present the first self-assembled substrate that yields a structural color change directly at the site of disassembly. The small optical path length of the substrate (~nm) may be better suited for microfluidic integration in comparison to suspended nanoassembly networks that require larger pathlengths (~cm) for colorimetric changes to be observable [3].

A substrate able to visually detect protease activity was designed as a proof-of-concept to demonstrate the structurally induced, scattered color shift of the biosensor. We believe that protease diagnostics are greatly underutilized in clinical settings and we present the use of scattered light from coupled gold nanoparticle assemblies and their subsequent disassembly via proteolysis as a colorimetric assay to detect protease activity. Cleavage of the nanoassemblies disengages the plasmon coupling between nanoparticles and shifts the observed DF scattered light from orange to green. (Fig. 1a) This model system is not only limited to protease activity monitoring, but is applicable to the detection of numerous biomolecules.

EXPERIMENTAL

Gold nanoparticles (AuNPs) were first deposited onto APTES (3-aminopropyltriethoxysilane) functionalized glass microscope slides. The negatively charged AuNPs, which served as the core of the nanoconstruct, were electrostatically immobilized onto the positively charged surface of the APTES glass slide through Coulombic attraction. (Fig. 1b, left) Next, a biotinylated ten amino acid long peptide substrate (biotin-GGRGDGKGGC-OH), cleavable by the serine protease trypsin, was incubated on the substrate. The cysteine residue located at the C-terminus of the peptide enabled the thiol-mediated adsorption of the peptide onto the surface of the core nanoparticle. Streptavidin conjugated satellite nanoparticles were then introduced and bound to the biotinylated N-terminus of the peptide for controlled, directional self-assembly. (Fig. 1c) Incubation of methoxy polyethylene glycol succinimidyl valerate (mPEG-SVA, MW = 2 kDa) on the substrate before satellite deposition blocked the microscope slide such that the satellites only attached to the core nanoparticles. (Fig. 1b, right) This essential step prevented the satellites from nonspecifically adsorbing onto the substrate and facilitated a reversible color shift. It should be noted that the linking peptide chain could be replaced by a variety of other biomolecules such as an oligonucleotide or aptazyme for diverse analyte identification. All substrates were stored in deionized (DI) water to prevent capillary remodeling caused by evaporation. The substrates remained optically active after 28 days when stored in DI water at 4°C.

Pairs of slides were created for each geometrical combination and analyzed under DF with an inverted microscope after each assembly step. A spectrophotometer measured the far-field spectral scattering from the substrate and data taken from five random positions on each substrate were averaged to determine peak position and full width at half maximum (FWHM). Core AuNPs were deposited at high densities, ~33.1 cores /g70m2 for 50 nm AuNP cores, enabling easy visualization of the scattered color yet substrates were not overly dense, which would cause unfavorable plasmonic coupling between core particles. Four different core diameters were investigated. 30 nm and 50 nm cores scattered...
green in comparison to the lime-green and yellow colors 80 nm and 100 nm cores respectively scattered. (Table 1) Additionally, the two larger core sizes have broader peakwidths (FWHM). Core sizes with green scattering, which is the most sensitive color to the human eye, are optimal as their plasmon peak will be red shifted upon satellite attachment and plasmon coupling. The red shift for assemblies with a large core size cannot be visually discerned.

**Table 1. Scattering Properties of Core AuNPS**

<table>
<thead>
<tr>
<th>Core AuNP Size [nm]</th>
<th>Peak Position [nm]</th>
<th>FWHM [nm]</th>
<th>Scattering Cross Section $\sigma_1$ [$\text{nm}^2$]</th>
<th>Scattering Efficiency $Q_s$</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>557.4</td>
<td>97.5</td>
<td>0.94</td>
<td>0.0013</td>
</tr>
<tr>
<td>50</td>
<td>560.8</td>
<td>84.6</td>
<td>18.31</td>
<td>0.0093</td>
</tr>
<tr>
<td>80</td>
<td>570.1</td>
<td>115.3</td>
<td>249.90</td>
<td>0.0497</td>
</tr>
<tr>
<td>100</td>
<td>569.9</td>
<td>110.1</td>
<td>806.05</td>
<td>0.1026</td>
</tr>
</tbody>
</table>

**THEORY**

An important property for core size is the scattering cross section ($\sigma_1$). When a particle radius ($r$) is much smaller than the wavelength of light ($r \ll \lambda$), Rayleigh theory states $\sigma_1 \propto \frac{r^6}{\lambda^4}$. Thus, core sizes of 30 nm and smaller have a small scattering cross section, resulting in dimly scattered green light which is difficult to visualize. (Table 1) The calculated scattering cross sections ($\sigma_1$) and scattering efficiencies ($Q_s$) in Table 1 were determined using Mie theory. The scattering efficiency is a proportionality parameter that relates the effective scattering cross section to the actual geometric cross section $\sigma_1 = \frac{\pi r^2 Q_s}{\lambda^4}$. The optimal core size, determined by its spectral peak position and scattering cross section, is 50 nm.

**RESULTS AND DISCUSSION**

The serine protease trypsin, known to cleave the carboxyl side of lysine and arginine, was used to initiate substrate disassembly. (Fig. 1d) Trypsin (2.5 g/L) was pipetted directly onto the substrate at room temperature (25°C) and produced a complete color change in less than 40 minutes as seen in the images captured by a true-color charge-coupled device (CCD) camera. (Fig. 2) Figures 2a, 2b, and 2c are time-lapse images of a substrate at 0 min, 30 min, and 60 min after trypsin exposure. The white spots observable in Fig. 2c are believed to be scattering from mPEG-SVA aggregates that accumulated during surface passivation. A representative DF spectrum depicts a colorimetric shift greater than 70 nm for a substrate with 50 nm cores and 50 nm satellites. (Fig. 2d) Modified Gaussian curves have been fitted to the raw data. A representative SEM image of a 100 nm core with 10 nm satellites is shown in Fig. 2e. Core sizes larger than 100 nm scatter light greater than 600 nm and satellite disassembly is indiscernible. Thus, larger sized core particles were eliminated from the study. Radiation damping has been shown to be responsible for red shifts in both the near- and far-field scattering from nanoparticles as they increase in size [4]. The nanoassembled substrate, before and after trypsin-mediated disassembly, is shown under bright field illumination in Fig. 2f. The short path length of the monolayer of core-satellites does not scatter enough of the incident light for a colorimetric change to be noticeably observed under bright field.

A large-scale characterization study was performed for the assembly and disassembly of an array of nanoconstructs. (Fig. 3) Core sizes ranged from 30 nm to 100 nm and satellite sizes ranged from 10 nm to 50 nm. Satellites larger than 50 nm are difficult to conjugate with streptavidin as the surface-to-volume ratio is large and the colloid becomes unstable during physio-adsorption of the streptavidin onto the AuNP surface. Peak shift upon assembly, defined as: $\Delta \lambda_{\text{peak}} = \lambda_{\text{peak,core+sat.}} - \lambda_{\text{peak,core}}$, was first studied. The maximum attained assembly red shifts were approximately ~70 nm with combinations of 50 nm and 30 nm AuNPs. (Fig. 3, top) Of all the satellite sizes, 30 nm satellites consistently produced a large peak shift. This behavior seems contrary to intuition as 50 nm satellites cause a larger structural change than 30 nm satellites. Limited...
streptavidin conjugation onto the larger 50 nm AuNPs may have resulted in fewer satellites capable of binding to the core particles. Next, the peak shift upon disassembly was analyzed. (Fig. 3, bottom) As before, combinations of 30 nm and 50 nm AuNPs provided the largest blue shift, with 30 nm core and 50 nm satellite assemblies causing a maximum shift of 48.3 nm. The assembly red shift is not completely reversed, which may be caused by nonspecific aggregation of the satellites to the substrate and irreversible core-satellite attachment. Overall, Fig. 3 reveals that core-satellite combinations of 30 nm and 50 nm result in the largest spectral peak shifts.

The second half of the characterization study analyzed the FWHM broadening during nanoconstruct assembly and narrowing during disassembly. (Fig. 4) Analysis of the FWHM is necessary to maximize the signal-to-noise ratio during biosensing as large FWHM increases may cause disassembly blue shifts to be indiscernible. The change in the bandwidth upon assembly, defined as $\Delta \text{FWHM} = \text{FWHM}_{\text{core+satellite}} - \text{FWHM}_{\text{core}}$, was a maximum, 75.9 nm, for the 50 nm core and 30 nm satellite assembly. (Fig. 4, top) Other combinations of 30 nm and 50 nm AuNP produced ~56 nm in bandwidth broadening. The maximum bandwidth narrowing was again obtained by the 50 nm core and 30 nm satellite. (Fig. 4, bottom) Maximizing the narrowing of the bandwidth during disassembly is essential such that the peak shift can be easily resolved by human eye. Ultimately larger satellite sizes should lead to greater FWHM broadening. Similar to Fig. 3, 30 nm satellites consistently produced the greatest fluctuations in bandwidth for both assembly and disassembly.

In order to quantify the overall biosensing capability of each nanoassembly variation, we propose the use of a figure of merit (FOM) for detecting plasmon peak shifts

$$FOM = \frac{\Delta \lambda_{\text{peak}}}{\langle \text{FWHM} \rangle}$$

where $\langle \text{FWHM} \rangle$ is the average of the two FWHM values (4). The FOM takes into account both the peak shift and plasmon bandwidth, quantifying the assemblies’ ability to provide a resolved and observable colorimetric change. The experimentally obtained FOM values are shown in Table 2 for the nanoassembly variations we have studied. The substrate with 30 nm cores and 50 nm satellites has the highest FOM value, 0.371. However, the small scattering cross section of a 30 nm core AuNP made this substrate very difficult to observe by naked eye. The second and third nanoassemblies with the next highest FOM values, 50 nm cores with 30 nm and 50 nm satellite sizes, yielded spectacular color shifts for our given experimental setup. (Fig. 2) These two combinations of nanoassembly are optimal.

<table>
<thead>
<tr>
<th>Core Size</th>
<th>10 nm</th>
<th>30 nm</th>
<th>50 nm</th>
<th>80 nm</th>
<th>100 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 nm</td>
<td>0.10</td>
<td>0.26</td>
<td>0.37</td>
<td>0.10</td>
<td>0.08</td>
</tr>
<tr>
<td>50 nm</td>
<td>0.05</td>
<td>0.28</td>
<td>0.25</td>
<td>0.09</td>
<td>0.03</td>
</tr>
<tr>
<td>80 nm</td>
<td>0.01</td>
<td>0.28</td>
<td>0.09</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>100 nm</td>
<td>0.07</td>
<td>0.10</td>
<td>0.09</td>
<td>0.03</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Figure of Merit for Various Core-Satellite Assemblies

CONCLUSION

In summary, we propose a method of biomolecular detection that is observable by naked eye in DF and is well suited for microfluidic integration. The proof-of-concept substrate was constructed by the self-assembly of core-satellites onto glass and the color shift was used for detecting the protease trypsin. Various combinations of nanoassemblies were optically characterized and a FOM was determined for each. Nanoassemblies with 50 nm cores and either 30 nm or 50 nm satellites were found to be optimal. To the best of our knowledge, this plasmonic biosensor is the first self-assembled substrate that yields a structural color change directly at the site of disassembly. This method may enable robust POC diagnostic devices capable of diverse species detection.

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REFERENCES


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