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Aging Induced Ag Nanoparticle Rearrangement under Ambient Atmosphere and Consequences for Nanoparticle-Enhanced DNA Biosensing

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Abstract

Localized surface plasmons of metallic nanoparticles can strongly amplify the magnitude of the surrounding electric field. This in turn enhances fluorescence from nearby fluorophores. However, little is known regarding how time-dependent changes in nanoparticle structure due to exposure to the ambient environment affect their behavior in plasmonic devices. Here, we report the interesting finding that the aging of a nanostructured Ag substrate in ambient atmosphere markedly improves the fluorescence signal of a plasmonic-based DNA detection system. The effect can be observed with an exposure time as short as two days, and a nearly 17-fold signal enhancement can be achieved with 30 days of aging. Analysis of substrate surface topography by atomic force microscopy (AFM) reveals a substantial change in nanoparticle morphology as the substrates age despite being covalently attached to a solid dry substrate. Nanoparticle morphological changes also manifest in extinction spectra. This process can be further accelerated by light. Together, our findings address the important question of Ag nanoparticle stability over time and its potential ramifications for plasmon-enabled sensors. They also imply that nanoparticle aging may be used strategically to tune nanoparticle size and geometry and plasmon spectrum, which may be beneficial for studies on plasmonics as well as sensor optimization.

Keywords

Ag nanoparticles; aging; nanoparticle coarsening; biosensor; metal enhanced fluorescence

INTRODUCTION

The ability of metal nanomaterials to strongly modulate the local electric field has led to their introduction into many photonic systems, including photovoltaics, light emitting diodes (LED), electronics, and sensors.^{1-2,3,4,5} Metal nanomaterials have found a particularly important area of application in biosensor development.^{6,7} Unlike bulk metals, metal nanoparticles display a strong UV-vis extinction band, resulting from the collective

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SUPPORTING INFORMATION AVAILABLE: Analysis of particle size and number as a function of time; AFM imaging and DNA detection performance of substrates following prolonged aging (3 pp.). This material is available free of charge via the Internet at <http://pubs.acs.org>.

oscillation of free electrons in resonance with the incident light, an effect known as localized surface plasmon resonance (LSPR).⁸⁻⁹ The large electric field localized at the metal surface enables metal nanoparticles to dramatically enhance both the fluorescence (metal-enhanced fluorescence, or MEF) and Raman signals from neighboring fluorophores and Raman labels, making them ideal candidates for incorporation into biosensors.¹⁰⁻¹¹⁻¹²⁻¹³⁻¹⁴⁻¹⁵ In addition, the plasmon extinction band is tunable based on nanoparticle size and shape, and has a high sensitivity to the local dielectric constant. Thus metal nanoparticles can also be incorporated into LSPR sensors in which molecular recognition is observed through changes in extinction spectra.¹⁶⁻¹⁷

As the number of sensing applications employing metal nanoparticles continues to increase, it is crucially important to understand how the environmental stability of metal nanomaterials (or lack thereof) affects detection precision and system reliability. Among different metals, nanoparticulate Ag has been the most prominently studied element in the field of surface enhanced spectroscopies owing to the very large natural plasmonic enhancement for Ag, coupled with its ease of handling and low cost.¹²⁻¹⁸ However, there is a perception that surface oxidation or other aging processes of metals can rapidly degrade a system's performance, given the fact that Ag rapidly oxidizes in ambient air.¹⁹⁻²⁰ Special care for Ag nanoparticle-based materials, such as storage under vacuum, is often taken as a precaution to prevent oxidation.²¹ Non-oxidative structural rearrangements of Ag nanoparticles on surfaces or in solution are also known. For example, Ag clusters were observed to aggregate spontaneously on metal surfaces *via* diffusion of adatoms at temperatures at or below room temperature in ultrahigh vacuum.²² Ostwald ripening (a process whereby smaller particles shrink, providing material which is drawn to larger particles, causing them to grow further) of Ag nanoparticles on metal surfaces driven by chemical potential differences between different sized nanoparticles has also been reported.²³⁻²⁴⁻²⁵ Structural rearrangement of Ag nanoparticles on tin oxide and graphite surfaces in solution through electrochemical Ostwald ripening has been demonstrated,²⁶ and photo-irradiation with auxiliary electric fields has been shown to induce Ag nanoparticle rearrangement on glass substrates in solution.²⁷ Other groups have described structural conversion of dispersed Ag nanoparticles from spheres to prisms in solutions utilizing visible light in resonance with the surface plasmon wavelength.²⁸⁻²⁹⁻³⁰⁻³¹⁻³²

Despite the many findings of spontaneous or light-stimulated Ag nanoparticle morphological rearrangements over time, the effect of exposure to an oxidizing environment (ambient atmosphere) on MEF-dependent sensor performance is unknown. Therefore, to begin to understand and potentially control such processes, we examined nanoparticle aging and rearrangement in an ambient oxidative environment in the context of a label-free (or "self-labeled") MEF-based DNA sensor. The DNA sensor employed in our studies relies on the behavior of DNA hairpin probes immobilized on a metal surface (Figure 1). Initially implemented on planar Au films,³³⁻³⁴⁻³⁵⁻³⁶⁻³⁷ a fluorophore-tagged, self-annealed DNA probe is first immobilized on a metal substrate *via* a thiol-metal bond. Hairpin formation places the fluorophore in close proximity to the metal surface, resulting in fluorescence quenching *via* energy transfer.³⁸ Introduction of target DNA to the system results in hybridization with the immobilized probe DNA, concomitantly unwraps the hairpin, and restores fluorescence. We recently demonstrated that nanostructured Ag substrates can substitute for planar Au in this DNA detection system,³⁹ providing both fluorescence quenching of the immobilized hairpin probe, and strong MEF in the presence of target complementary DNA. As sensor performance critically depended on nanoparticle size and density, it struck us that this could also constitute a useful probe for the effects of nanoparticle aging. We demonstrate below that these studies have allowed us to significantly improve sensor performance. Specifically, a nearly 17-fold fluorescence enhancement was observed from substrates aged 30 days in an ambient environment relative to as-prepared

substrates. We observe that the aging process results in a change in the structure of the nanoparticles on the substrate, and a concomitant 30 nm red-shift in the extinction spectrum. These observations provide new and useful strategies for tuning plasmonic spectra, and for probing structural changes in metal nanoparticles indirectly through the fluorescence signal.

EXPERIMENTAL SECTION

Substrate Preparation

A glass microscope slide (VWR) was first diced into 5×10 mm chips by hand. Next, the glass chips were cleaned by soaking them in a piranha solution (sulfuric acid: hydrogen peroxide; 3:1) for 15 min (Caution, piranha solution is highly caustic and can react explosively with organic materials), washed extensively with glass distilled, deionized (DDI) water and immediately dried under nitrogen gas. An additional cleaning step was carried out by soaking the glass chips in a 10 M NaOH solution for 5 min, followed by rinsing with DDI water, and finally drying under nitrogen gas. The cleaned glass chips were then functionalized with methoxysilane bearing a thiol group, which was accomplished by incubating them in a solution composed of 1% 3-mercaptopropyl trimethoxysilane (MPTS), 95% methanol, and 4% 1 mM acetic acid at room temperature for 30 min. Next, the silanized glass chips were washed by sonication (300-W Vibracell probe sonicator, Sonic & Materials Inc.) in a 95% ethanol : 5% water solution for 2 min, and dried under nitrogen gas. Coating of Ag nanoparticles on the silanized glass chips was achieved by incubating them in a solution of 10 mM AgNO_3 in dimethylformamide (DMF) at room temperature in the dark for 1 hr.⁴¹ The resulting Ag nanoparticle-coated glass chips were then washed by sonication in a 95% ethanol: 5% water solution for 4 min, and dried under nitrogen gas. The Ag nanoparticle coated substrates were then stored under ambient atmosphere (average temperature: 24°C, average humidity: 36.8%), in the dark for different periods of time (0, 2, 5, 9, 12, 16, 19, 30 days) prior to DNA immobilization. For the non-oxidative environment study, the substrates were stored in a glove box (Vacuum Atmosphere Company) filled with N_2 gas (average temperature: 24°C, average humidity: 0%) and allowed to age for 30 days.

Self-Assembly

Self-assembly of DNA hairpin probes on nanostructured Ag substrates was accomplished by incubating each Ag substrate in a solution consisting of 300 nM probe DNA and 60 nM mercaptopropanol in buffered saline (500 mM NaCl, 20 mM cacodylic acid, and 0.5 mM ethylenediaminetetraacetic acid (EDTA), pH=7.0) for 2 hr at room temperature in the dark. Probe DNA (Midland) has a sequence of 5'-TCG TTA GTG TTA GGA AAA AAT CAA ACA CTC GCG A-3' and is conjugated with a trityl-thiol group and a tetramethylrhodamine (TAMRA, Ab_{max} : 543 nm, Em_{max} : 571 nm) fluorophore at the 5' and 3' ends, respectively. Next, non-specifically absorbed DNA probes were removed by washing the substrates in boiling DDI water for 30 s. Substrates were then air dried in the dark at room temperature for 45 min. Hairpin formation was promoted by immersing dried substrates in buffered saline in the dark at room temperature for another 45 min. Pre-hybridization fluorescence intensity was acquired after removal from the saline solution. Subsequently, the probe immobilized Ag substrates were incubated in a 2.5 μM label-free DNA target solution overnight followed by post hybridization fluorescence intensity measurement. Target DNA (IDT) has a sequence complementary to the probe DNA (5'-TCG CGA GTG TTT GAT TTT TTC CTA ACA CTA ACG A-3').

Imaging Acquisition and Data Analysis

Fluorescence images were acquired by using an epifluorescence microscope (Olympus BX-60) equipped with a thermoelectrically (TE) cooled charge coupled device (CCD). Sample substrates were excited with incident light from a Hg lamp (100-W), which was

filtered with an excitation bandpass filter (531 ± 20 nm), reflected by a dichroic mirror, and guided through a $10\times$ objective lens. The emitted light was collected by CCD after the light was reflected from the sample, guided through the objective lens, the dichroic mirror, and a bandpass filter (593 ± 20 nm). Fluorescence images were then analyzed using ImageJ software.⁴⁰ To analyze particle size distributions, AFM images were first converted to 8-bit images followed by threshold adjustment. Each image was adjusted with fixed threshold for comparison purpose. Processed images were then analyzed with “Analyze Particles” function from ImageJ and the information made available was then binned in 500 nm^2 intervals using histogram command in Matlab.

Substrate Characterization

Extinction spectra of nanostructured Ag substrates were measured using a Perkin-Elmer Lambda 950 UV/vis/NIR spectrophotometer over wavelengths ranging from 330 nm to 750 nm. AFM images of the Ag substrates were measured in air using a Digital Instruments Nanoscope IIIa operated in tapping mode using a Si tip (300 kHz, 40 N/m). Substrate roughness measurements were made offline using Digital Instrument (DI) software.

TAMRA Characterization

The absorption spectrum of TAMRA was acquired using a Shimadzu UV- 1601PC spectrophotometer over wavelengths ranging from 450 nm to 600 nm. The emission spectrum of TAMRA was collected with a modular Acton Research fluorimeter equipped with a PMT (photomultiplier tubes) detector over wavelengths ranging from 530 nm to 750 nm at an excitation wavelength of 500 nm.

RESULTS AND DISCUSSION

To study nanostructured Ag substrate aging under ambient atmosphere and its potential effect on biomolecule detection, substrates were prepared at day 0 and examined for target DNA-dependent fluorescence at 0, 2, 5, 9, 12, 16, 19, and 30 days after substrate preparation. Ag nanoparticles were formed by AgNO_3 reduction in dimethylformamide (DMF) in the presence of a mercaptosilane-treated glass substrate, allowing their *in situ* deposition.⁴¹ Prior to DNA probe immobilization, the substrates were allowed to age under ambient atmosphere (average temperature: 24°C , average humidity: 36.8%) in the dark. Hairpin DNA probes were then immobilized on the aged substrates and the resulting sensors were exposed to target complementary DNA as previously described.³⁹ Figure 2 depicts the sensor responses from pre- to post-hybridization versus aging time. While there was little variation in pre-hybridization intensity, the post hybridization signal improved dramatically over the 30-day aging period under ambient air. For instance, the post-hybridization fluorescence intensity increased from 1793 ± 664 (a.u) at day 0 to 35626 ± 6518 (a.u) at day 30, corresponding to a nearly 17-fold signal enhancement. Given the known ability of Ag to readily oxidize,^{19,20} this observation was contrary to the expectation that prolonged substrate incubation under ambient atmosphere would degrade detection performance.

In order to develop a structural understanding of the nanoparticle aging process and its effect on detection performance, we first turned our attention to the extinction spectra of the aged substrates. As shown in Figure 3, the spectrum at day 0 (as-prepared substrate) displays a sharp peak at 398 ± 5 nm ($N=5$). This peak red-shifts to 426 ± 2 nm ($N=3$) over a 30-day period. Peak widths also increase as a function of aging time: the full width at half maximum (FWHM) of the spectrum increased from ~ 96 nm (day 0) to ~ 127 nm (day 30). Electrodynamics theory indicates that both phenomena can result from modulation of the dielectric constant of the embedding medium, changes in cluster size, or changes in cluster morphology.^{8,9,42,43}

We next investigated substrate surface topographies as a function of substrate aging by atomic force microscopy (AFM) to elucidate the contributing factors for spectral evolution over time. Inspection (Figure 4) and particle analysis (Supporting Information S1) of the images both reveal a decrease in the number of smaller particles, an increase in the number of larger particles and an overall decrease in particle number. As shown in Figure 4a, particles are largely spherical at day 0, with an apparent diameter of ~16 nm and a root mean square (RMS) roughness of 2.2 nm. Particles undergo a readily observable increase in size between day 0 and day 12, while largely retaining their spherical shape (Figures 4b and 4c). By day 30 a more pronounced transformation has obviously taken place (Figure 4d): in addition to further increases in particle size, many are rodlike. These rodlike structures may be the result of incomplete coalescence (pre-spheroidization) between two or more particles.^{44,45} The progression of particle evolution, specifically the growth of larger particles at the expense of smaller particles, is consistent with the Ostwald ripening process described above. This led us to postulate that the mechanism of particle rearrangement observed in our system to be a result of Ag oxidation under ambient air and subsequent deposition onto the nearby Ag nanoparticles where greater stability is gained. Ag⁺ ions that are formed after oxidation travel to neighboring nanoparticles potentially through a thin moisture layer that is normally formed on the nanostructured substrate by water condensation under ambient atmosphere.^{46,47}

AFM images of 60 and 480 day-old substrates illustrate the presence of both rodlike structures and larger particles on the substrate surface (Supporting Information S2). Furthermore, examination of the 480 day-old Ag substrates demonstrates that the substrate remains responsive, albeit less sensitive as compared to the 30 day-old Ag substrate (Supporting Information S3). We note that no special care was taken to protect these substrates from ambient dust, chemical vapors, etc. during storage. Therefore, numerous factors in addition to the changes in particle size may have contributed to the reduction in detection sensitivity observed from the 480-days old substrate, any of which may have contributed to substrate degeneration. In particular, prolonged aging may have resulted in extensive oxidation, thereby impeding the Ag-thiol bond formation. This finding indicates the importance of storing the nanostructured metal substrates in a non-oxidative (or a more appropriate) environment for long-term use.

The influence of the dielectric constant of the embedding medium on extinction spectra of metal nanoparticles has been demonstrated extensively,^{20,48,49,50} and could also contribute to the changes in device performance. In particular, exposure of Ag nanoparticles to oxygen or ambient air results in spectral red-shifts and broadening, stemming from a depressing surface charge in turn caused by the change in the dielectric medium.⁹ The resultant extinction spectrum change can be well characterized by electrodynamic calculations modeling core-shell nanostructures, in which the shell has the dielectric constant of the embedding medium (oxide) and the core remains pure Ag.⁴³ Unfortunately, a direct quantification of oxide content on our Ag substrates by analytical techniques such as Energy Dispersive X-ray Spectroscopy (EDS) and X-ray Photoelectron Spectroscopy (XPS) is problematic due to the low conductivity of the Ag substrates.⁵¹ Since the Ag substrates were aged in ambient air, oxide formation on the Ag substrate was almost inevitable.⁵² While the contribution of this oxide formation could not be evaluated explicitly, the direct experimental examination of changes in nanoparticle size and shape can account for our observations and thus any dielectric effects are probably small.

As mentioned earlier, the presence of visible and UV light has been implemented to assist metal particle reformation on both conductive and dielectric substrates. Particles of a particular geometry can be tailored from initial spherical or pseudospherical seeds based on the wavelength of the light source.^{27,28,29,30,31,32} To examine the effect of light on

substrate aging, the freshly fabricated Ag substrates were left on the lab bench and exposed to ambient light (fluorescent tubes located on the laboratory ceiling, 4100k fluorescent lamp, F32T8/FL841, 32W) for 24 hr per day for a period of 30 days. The intensity of the incident light on the substrates was measured to be $\sim 0.02 \text{ mw cm}^{-2}$ with the use of a light meter (Gossen Scout 3, Japan). The light-exposed Ag substrates show a diminishing extinction amplitude but nearly identical peak position ($\sim 428 \text{ nm}$) as compared to the substrate aged in the dark for 30 days (Fig. 5). The diminishing amplitude seen with the presence of ambient light (as compared to the substrates that were aged in the dark) is possibly a result of accelerated Ag dissolution caused by the ambient light. The AFM image of the substrate (Fig 6B) illustrates a decrease in particle number ($\sim 60\%$ decrement), increase in particle size, and a decrease in particle height. RMS roughness of the substrate decreased from 2.2 nm at day 0 to 1.5 nm at day 30. Particle morphology stayed primarily spherical, with a few elongated structures ($< 0.5\%$).

The effect of ambient light on particle coalescence is currently unclear. It is possible that ambient light facilitates Ag^+ deposition onto the larger particles *via* the plasmon excitation mechanism (the emission spectrum of the fluorescence tube used in this study displays two peaks, positioned at ~ 405 and 436 nm , respectively), resulting in an increase in the rate of large particle growth.⁵⁴ Clearly, the dried and air-exposed substrates we describe in this study are very different from the experimental conditions previously employed to examine light-accelerated Ag nanoparticle remodeling,²⁷ but it is possible that sufficient moisture remains on the substrate surface to permit some of the same processes to occur. To test this hypothesis, we replaced the oxidative environment (air and humidity) with N_2 gas. We placed the freshly prepared Ag substrates in a glove box (Vacuum Atmosphere Company) filled with N_2 that allows for a rigorous control of humidity (humidity: 0%). The substrates were also exposed under a fluorescent tube. The resultant extinction spectrum shows no change in extinction amplitude (Figure 5), indicating the significant role of moisture on damping of the extinction amplitude and structural rearrangement. It also shows that light alone in a non-oxidative environment is insufficient to induce a significant spectral change over time. Although the N_2 aged group (30 days) appears to show a peak position slightly to the right of the 0-day group, the difference is within the chip-to-chip standard deviation ($398 \pm 4.6 \text{ nm}$, $N=5$). DNA detection performed on substrates treated with N_2 (humidity: 0%) in the dark for 30 days showed no improvement of fluorescence signal as compared to as-prepared substrates (data not shown).

Besides photonic energy derived from auxiliary sources such as fluorescent tubes, certain chemical additives also acquire the ability to drive particle rearrangement. For instance, exposure to excess chalcogen elements such as oxygen and sulfur can dramatically increase the propensity of the Ag nanoparticles to coalesce.^{55,56} In contrast, additives such as *n*-alkanethiols can protect the metal surfaces from corrosion.⁵⁷ To investigate the question of whether attached DNA hairpin probes could alter the rate of particle aging, we examined the detection responses of two 12-day aged substrates that were pre-functionalized with DNA hairpin probes at day 0, and compared their performances to the responses of the substrates that received no ageing treatments (Supporting Information S4). In contrast to the unfunctionalized substrates, no significant improvement in fluorescence signals was observed from the aged substrates as compared to the as-prepared substrates. These results are consistent with the DNA hairpin probes acting as protective molecules, preventing structural rearrangement of the underlying Ag nanoparticles.

In experiments discussed thus far, we have addressed the effect of substrate aging on nanoparticle coarsening under the influences of ambient air and ambient light. How does this aging-induced structural change improve the fluorescence detection signal as demonstrated in Figure 2? The improving system performance over time can be attributed to

both plasmonic and “lightning rod” effects. Studies have shown that the level of fluorescence enhancement is strongly influenced by the spectral overlap between the plasmonic wavelength and the absorption/emission bands of the fluorophores.^{10,58,59,60,61} Substrate aging under ambient atmosphere induced spectrum red-shifts as a result of nanoparticle coarsening (Figure 3 and Figure 4), which brought the plasmonic wavelength closer to the absorption/emission band of the TAMRA molecule (λ_{max} : 543 nm, λ_{em} : 571 nm) and resulted in a greater fluorescence enhancement over time. Another possible contributing factor for the dramatic enhancement of fluorescence signals observed over time is the much higher local electric fields enhanced by the surface irregularities, sharp corners, and small aggregates that are formed on the substrates.^{62,63} Both elliptical nanowires and arbitrary particle shapes with sharp edges have demonstrated much higher levels of localized electric fields surrounding the structures, which can enable much more pronounced enhancing phenomena.

In previous work, we observed that increasing Ag exposure to the mercaptosilane-treated glass substrates also shifted the extinction spectra closer to the excitation/emission band of TAMRA. However, a decrease in detection performance with an equal amount of DNA probe hairpin and spacing agent was observed.³⁹ How does one reconcile those results with the observations reported herein? The reduction in detection performance seen in the previous study stemmed mainly from an increase in pre-hybridization signal with increasing Ag exposure time, a result of steric crowding or continuum background emission of the rough Ag surface. As demonstrated in this study, substrate aging induces particle reformation, but this occurs in a manner such that enough void space remains between particles on the substrate to alleviate steric crowding and continuum background emission from the Ag surfaces. A rough estimation of the particle surface area change from day 0 to 30 days of aging showed only limited changes over time (an approximate 4.5% increase in surface area). Therefore, it is unlikely that steric crowding effects of DNA probes play more than a minimal role in the observed changes in the detection performance over time. In addition, our previous findings suggest that the steric crowding phenomenon primarily manifests in an increase in the pre-hybridization signals, as hairpin probes are unable to achieve a configuration suitable for full quenching of the fluorophore. Thus, one would expect to observe a noticeable increase in pre-hybridization signals over time if steric crowding is taking place. This was not observed.

This report summarizes preliminary studies on the effect of Ag nanomaterial stability on the performance of a fluorescence based DNA biosensor. Our findings demonstrate the dependence of fluorescence signals on substrate aging time. A lengthening incubation (30 days) of nanostructured Ag substrates under ambient atmosphere induced particle coalescence, despite covalent attachment of particles to a glass substrate. Ag nanoparticle coalescence red-shifted the extinction spectrum and generated a nearly 17-fold increase in fluorescence response. We hypothesize that the improvement in the fluorescence response observed after aging is due to both plasmonic and lightning rod effects.

We attribute the mechanism of particle rearrangement under ambient conditions to oxidative dissolution of Ag, and subsequent deposition of Ag^+ onto nearby Ag nanoparticles as a reductive reaction. Based on electrode potential calculations, smaller Ag nanoparticles are more easily oxidized as compared to larger particles.²⁶ Ag^+ ions formed on the smaller particles under ambient conditions can potentially travel to the neighboring larger particles with the assistance of the moisture layer on the substrate. This process is driven by the electric field gradient since larger nanoparticles have a partial negative charge at electrical equilibrium. To reestablish electrical equilibrium after ion deposition, the larger nanoparticle then accepts an electron from touching smaller nanoparticles. Inclusion of ambient light in conjunction with ambient air in the system further facilitated particle coalescence.

Replacement of the oxidative environment (ambient air) with N₂ gas in the presence of ambient light diminished the structural change significantly, indicating ambient air is necessary for structural rearrangement, and light alone is insufficient to initiate particle reformation.

CONCLUSIONS

A detailed prediction of metal nanostructures after aging treatments is extremely challenging, as many factors including particle coverage, temperature, impurity, additives, auxiliary energy source, and interactions with the substrate can influence interfacial energy and mass transfer greatly. Further work will be required to fully understand the impact of each of these factors on particle rearrangement. It is clear, however, that from the applications perspective (at least in the context of MEF-based biosensors), Ag nanoparticle substrate aging in ambient atmosphere does not necessarily degrade a system's performance, as one might expect. On the contrary, it improved the detection/fluorescence signal significantly. In conclusion, this work illustrates the importance of understanding a studied nanomaterial's stability over time. We anticipate the DNA biosensing system described herein will be of further analytical utility for probing structural changes in nanostructured metal substrates through the fluorescence signal.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Moulin E, Sukmanowki J, Schult M, Gordijn A, Royer FX, Stiebig H. *Thin Solid Films*. 2008; 516:6813–6817.
2. Coe S, Woo W-K, Bawendi MG, Bulovic V. *Nature*. 2002; 420:800–803. [PubMed: 12490945]
3. Ko SH, Park I, Pan H, Grigoropoulos CP, Pisano AP, Luscombe CK, Fréchet JM. *Nano Lett*. 2007; 7:1869–1877. [PubMed: 17547465]
4. Prasad, PN. *Nanophotonics*. New Jersey: Wiley-Interscience; 1998. Ch. 13.
5. Skrabalak SE, Chen J, Au L, Lu X, Li X, Xia Y. *Adv. Mater*. 2007; 19:3177–3184. [PubMed: 18648528]
6. Anker JN, Hall WP, Lyanders O, Shan NC, Zhao J, Van Duyne RP. *Nat. Mater*. 2008; 7:442–453. [PubMed: 18497851]
7. Taton TA, Mirkin CA, Letsinger RL. *Science*. 2000; 289:1757–1760. [PubMed: 10976070]
8. Willets KA, Van Duyne RP. *Annu. Rev. Phys. Chem*. 2007; 58:267–297. [PubMed: 17067281]
9. Kreibig, U.; Vollmer, M. Ch. 2. In: Toennies, JP.; Gonser, U.; Osgood, RM., Jr; Panish, MB.; Sakaki, H., editors. *Optical Properties of Metal Clusters*. Vol. 25. Berlin: Springer-Verlag; 1995.
10. Bharadwaj P, Anger P, Novotny L. *Nanotechnology*. 2007; 18:044017.
11. Lakowicz JR. *Anal. Biochem*. 2005; 337:171–194. [PubMed: 15691498]
12. Moskovits M. *Rev. Mod. Phys*. 1985; 57:783–826.
13. Nie S, Emory SR. *Science*. 1997; 275:1102–1106. [PubMed: 9027306]

14. Yonzon CR, Haynes CL, Zhang X, Walsh JT Jr, Van Duyne RP. *Anal. Chem.* 2004; 76:78–85. [PubMed: 14697035]
15. Aslan K, Zhang Y, Geddes CD. *Anal. Chem.* 2009; 81:4713–4719. [PubMed: 19432434]
16. Haes AJ, Van Duyne RP. *J. Am. Chem. Soc.* 2002; 124:10596–10604. [PubMed: 12197762]
17. Jain PK, Huang XH, El-Sayed IH, El-Sayed MA. *Acc. Chem. Res.* 2008; 41:1578–1586. [PubMed: 18447366]
18. Chan GH, Zhao J, Hicks EM, Schatz GC, Duyne RP. *Nano Lett.* 2007; 7:1947–1952.
19. Erol M, Han Y, Stanley SK, Stafford CM, Du H, Sukhishvili S. *J. Am. Chem. Soc.* 2009; 131:7480–7481. [PubMed: 19445502]
20. Yin Y, Li ZY, Zhong Z, Gates B, Xia Y, Venkateswaran S. *J. Mater. Chem.* 2002; 12:522–527.
21. Pribik R, Dragan AI, Zhang Y, Gaydos C, Geddes CD. *Chem. Phys. Lett.* 2009; 478:70–74. [PubMed: 20352080]
22. Röder H, Hahn E, Brune H, Bucher J-P, Kern K. *Nature.* 1993; 366:141–143.
23. Morgenstern K, Rosenfeld G, Comsa G. *Surf. Sci.* 1999; 441:289–300.
24. Wen JM, Chang SL, Burnett JW, Evans JW, Thiel PA. *Phys. Rev. Lett.* 1994; 73:2591–2594. [PubMed: 10057099]
25. Wen JM, Evans JW, Bartelt MC, Burnett JW, Thiel PA. *Phys. Rev. Lett.* 1996; 76:652–655. [PubMed: 10061513]
26. Redmond PL, Hallock AJ, Brus LE. *Nano Lett.* 2005; 5:131–135. [PubMed: 15792426]
27. Murakoshi K, Tanaka H, Sawai Y, Nakato Y. *J. Phys. Chem. B.* 2002; 106:3041–3045.
28. Callegari A, Tonti D, Chergui M. *Nano Lett.* 2003; 3:1565.
29. Jin RC, Cao YC, Hao E, Métraux GS, Schatz GC, Mirkin CA. *Nature.* 2003; 425:487–490. [PubMed: 14523440]
30. Xue C, Millstone JE, Li S, Mirkin CA. *Angew. Chem. Int. Ed.* 2007; 44:8588–8591.
31. Xue C, Métraux GS, Millstone JE, Mirkin CA. *J. Am. Chem. Soc.* 2008; 130:8337–8344. [PubMed: 18533653]
32. Wu X, Redmond PL, Liu H, Chen Y, Steigerwald M, Brus L. *J. Am. Chem. Soc.* 2008; 130:9500–9506. [PubMed: 18578529]
33. Du H, Disney MD, Miller BL, Krauss TD. *J. Am. Chem. Soc.* 2003; 125:4012–4013. [PubMed: 12670198]
34. Du H, Strohsahl CM, Camera J, Miller BL, Krauss TD. *J. Am. Chem. Soc.* 2005; 127:7932–7940. [PubMed: 15913384]
35. Strohsahl CM, Du H, Miller BL, Krauss TD. *Talanta.* 2005; 67:479–485. [PubMed: 18970192]
36. Strohsahl CM, Krauss TD, Miller BL. *Biosens. Bioelectron.* 2007; 23:233–240. [PubMed: 17512187]
37. Strohsahl CM, Miller BL, Krauss TD. *Nat. Protoc.* 2007; 2:2105–2110. [PubMed: 17853865]
38. Wang X, Tang Z, Yang CJ, Kim Y, Fang X, Li W, Wu Y, Medley CD, Cao Z, Li J, Colon P, Lin H, Tan W. *Angew. Chem. Int. Ed.* 2008; 47:2–17.
39. Peng H-I, Strohsahl CM, Leach KE, Krauss TD, Miller BL. *ACS Nano.* 2009; 3:2265–2273. [PubMed: 19585997]
40. Abramoff MD, Magelhaes PJ, Ram SJ. *Biophoton. Int.* 2004; 11:36–42.
41. Pastoriza-Santos I, Liz-Marzan LM. *Langmuir.* 1999; 15:948–951.
42. Kreibig U, Genzel L. *Optical Absorption of Small Metallic Particles. Surf. Sci.* 1985; 156:678–700.
43. Kelly KL, Coronado E, Zhao LL, Schatz GC. *J. Phys. Chem. B.* 2003; 107:668–677.
44. Nichols F, Mullins W. *J. Appl. Phys.* 1965; 36:1826–1835.
45. Zhang LH, Sui ML, Zhang L, Hu KY, Li DX. *Mater Sci Eng A Struct Mater.* 2004; 379:1–6.
46. Roark SE, Semin DJ, Rowlen KL. *Anal. Chem.* 1996; 68:473–480.
47. Thundat T, Zheng X-Y, Chen GY, Warmack RJ. *Surf. Sci. Lett.* 1993; 294:L939–L943.
48. Henglein A. *Chem. Mater.* 1998; 10:444–450.
49. Kreibig U, Gartz M, Hilger A. *Ber. Bunsenges. Phys. Chem.* 1997; 101:1593–1604.

50. Xu G, Tazawa M, Jin P, Nakao S, Yoshimura K. *Appl. Phys. Lett.* 2003; 82:3811–3813.
51. Tricoli A, Pratsinis SE. *Nat. Nanotechnol.* 2010; 5:54–60. [PubMed: 19946284]
52. Cai W, Zhong H, Zhang L. *J. Appl. Phys.* 1998; 83:1705–1710.
53. Lu J, Bravo-Suárez JJ, Takahashi A, Haruta M, Oyama ST. *J. Catal.* 2005; 232:85–95.
54. Millstone JE, Hurst SJ, Métraux GS, Cutler JI, Mirkin CA. *Small.* 2009; 5:646–664. [PubMed: 19306458]
55. Layson AR, Evans JW, Thiel PA. *Phys. Rev. B.* 2002; 65:193409.
56. Thiel PA, Shen M, Liu D-L, Evans JW. *J. Phys. Chem. C.* 2009; 113:5047.
57. Jennings GK, Yong T-H, Munro JC, Laibinis PE. *J. Am. Chem. Soc.* 2003; 125:2950. [PubMed: 12617662]
58. Stranik O, Nooney R, McDonah C, MacCraith BD. *Plasmonics.* 2007; 2:15–22.
59. Tam F, Goodrich GP, Johnson BR, Hala NJ. *Nano Lett.* 2007; 7:496–501. [PubMed: 17256995]
60. Chen Y, Munechika K, Plante IJ-L, Munro AM, Strabalak SE, Xia Y, Ginger DS. *Appl. Phys. Lett.* 2008; 93:053106-3.
61. Cade NI, Ritman-Meer T, Kwakwa KA, Richards D. *Nanotechnology.* 2009; 20:285201. [PubMed: 19546490]
62. Gersten J, Nitzan A. *J. Chem. Phys.* 1980; 73:3023–3037.
63. Kottmann JP, Martin OJF, Smith DR, Schultz S. *Chem. Phys. Lett.* 2001; 341:1–6.

Figure 1.

Working principle of the DNA detection system. (A) A fluorophore tagged DNA hairpin is first immobilized on a nanostructured Ag substrate *via* a thiol-metal bond. The hairpin brings the fluorophore in close proximity to the Ag substrate surface and the fluorescence is quenched due to energy transfer. (B) Introduction of a target DNA, of complementary sequence to the probe DNA. (C) Target DNA hybridizes with the probe DNA, brings the fluorophore away from the substrate surface and restores the fluorescence.

Figure 2.

Detection responses of the DNA hairpin probe immobilized-nanostructured Ag substrates versus aging time. Prior to DNA hairpin probe immobilization, Ag substrates were allowed to age under ambient atmosphere in the dark for 0, 2, 5, 9, 12, 16, 19, and 30 days. Fluorescence signals were obtained before (pre-hybridization) and after (post-hybridization) DNA target exposure to the probe immobilized-Ag substrates that were aged for certain periods of time. Gray and black bars represent pre- and post- hybridization signals, respectively. Results show that the detection performances vary significantly as a function of substrate aging time. Data are presented as mean $\pm$ standard deviation. CCD exposure time: 500 ms. N=5 (5 substrates). Statistical analysis was performed using one-way ANOVA with *tukey post hoc* test on post hybridization signals (MATLAB). (*) Significantly different from all the groups to the left.

Figure 3.

Extinction spectra of aged nanostructured Ag substrates obtained at different days (0, 2, 30, and 45 days) after substrate preparation. The figure is also embedded with absorption and emission spectra of the DNA probe fluorophore tetramethylrhodamine (TAMRA). Extinction spectrum of the Ag substrate displays a sharp peak position at 398 ± 5 nm ($N = 5$) nm at day 0, which is found to shift to 426 ± 2 nm ($N = 3$) nm over a 30-day period. These shifts are accompanied by spectrum broadenings, which can be results of changes in particle size, shape or dielectric environment. Shifts of the spectra brought them closer to resonance with the absorption and emission energies of TAMRA.

Figure 4. AFM images illustrate structural revolution of the nanostructured Ag substrate at (A) 0, (B) 2, (C) 12, and (D) 30 days after substrate preparation and storage in the dark and ambient atmosphere. Shapes of the particles evolved from mainly spherical at 0 day to a combination of spherical and rodlike structures at 30 days. Scale bar: 200 nm.

Figure 5.

Extinction spectra of an untreated nanostructured Ag substrate (purple), and aged nanostructured Ag substrates treated under different environmental conditions (N_2 +ambient light (humidity:0% - green), Air+ambient light (humidity:0% - pink) and Air-ambient light (humidity:36.8%) - blue) for 30 days. The uptick displayed in the spectrum (Air+ambient light) below 350 nm is due to the interband electronic transition in Ag.⁵³

Figure 6. AFM images of the (A) as-prepared nanostructured Ag substrate and (B) the nanostructured Ag substrate aged 30 days under ambient air and ambient light. Prolonged aging of the substrate in under ambient light and ambient air results in a decrease in particle number, an increase in particle size and a lower RMS roughness. Scale bar: 200 nm