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Letters to *Analytical Chemistry*

Silver Nanoparticles on a Plastic Platform for Localized Surface Plasmon Resonance Biosensing

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A cost-effective fabrication method for the preparation of localized surface plasmon resonance (LSPR) biosensors supported on plastics is described. The silver-nanoparticles-on-plastic sensor (SNOPS) was fabricated by chemically modifying the surface of a common plastic, polyethylene terephthalate (PET) to allow the efficient immobilization of Ag NPs. The LSPR of the SNOPS strip showed good sample-to-sample reproducibility. The analytical performance of the sensor strips for monitoring both thiol and protein adsorption, including bioaffinity, was examined. The limit of quantification to the adsorption of 11-mercaptoundecanoic acid was 500 nM and for the detection of streptavidin was ~9.5 nM. SNOPS can then be used as a cheap, versatile, and yet sensitive LSPR biosensor.

Localized surface plasmon resonance (LSPR) spectroscopy has attracted lots of attention from the bioanalytical community.[1] LSPR offers similar merits as the conventional propagating SPR but with some important advantages. For instance, they are more suitable to microchip integration, provide faster response time, and have much better spatial resolution.[2] Moreover, the LSPR wavelength can be tuned throughout the visible and near-infrared by a judicious choice of nanoparticle's size, shape, and material.[1d,3]

Suspensions of metallic nanoparticles (MNPs), especially Au NPs, have been used to detect DNA hybridization[4] and in immunoassays.[4b,5] However, chemical analysis using MNPs suspended in a liquid phase suffers from the inherent stability issues related to the colloidal system. Furthermore, the approach is not suitable for high-throughput analysis,[6] as is the case for MNPs immobilized on a solid support.[1a,2b,3b,7]

Many groups have vastly contributed to the development of LSPR sensors in planar substrates.[1a,2a,8,2b,9,2b] LSPR sensor chips on glass fabricated through the wet chemistry method have also been reported[3b,6,7b,10] and used for monitoring biotin−streptavidin (SA) binding,[1d,3b,6] antigen−antibody interactions,[7b] and to detect small molecules.[3b,10d,e] Glass has been the most popular supporting material for LSPR sensors platforms. Although glass is inexpensive; it is fragile, nonflexible, and relatively heavy. Efforts to develop an alternative supporting substrate, such as paper,[7a,11] for a LSPR-based sensor have been reported.

Polyethylene terephthalate (PET) is a flexible, light, and cheap transparent polymer. Biosensors supported on other polymer films have been widely reported in order to explore these attractive properties.[12] In the specific case of PET, it is a material that has been used as a substrate for transparent flexible displays, organic

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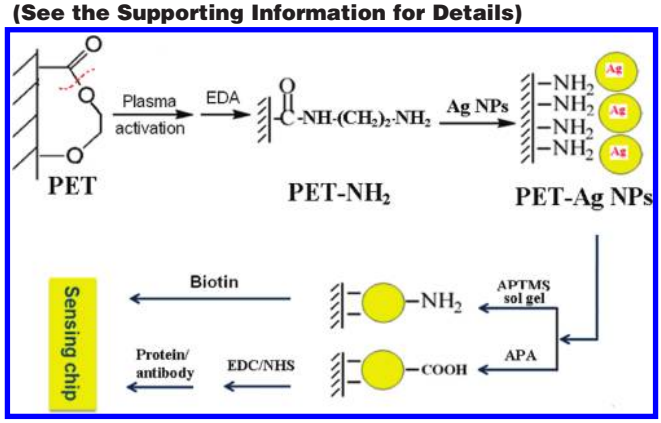
Scheme 1. Preparation of SNOPS Strip for Biosensing (See the Supporting Information for Details)



electronic devices, and biomedical devices.[13] Recently, PET has been used as a substrate for nanofabrication,[13a] protein microarrays,[14] and glucose biosensors.[15] Surprisingly, there is very limited efforts on using PET as a substrate to support LSPR-based sensors.[16]

Previously, we have reported on methods[17] to self-assemble MNPs on different solid supports for SERS detection. Here, we expand on those efforts by using PET as a solid support for Ag NPs self-assembly.

EXPERIMENTAL SECTION

The general procedure for the preparation of the silver-nanoparticles-on-plastic sensor (SNOPS) strips is summarized in Scheme 1. The PET surface was first activated using a plasma oven. The activated strip was then immersed in a solution of 1,2-ethylenediamine (EDA) to impart the amino functionality, turning the surface reactive toward MNPs, such as silver and gold. The amino-modified PET strip was then incubated in a suspension of Ag NPs. A systematic optimization of the experimental parameters, such as the concentration of EDA and the incubation time on the Ag NPs suspension, was performed and it is presented as Supporting Information. The PET strip containing Ag NPs was further modified by either amino (using (3-aminopropyl)trimethoxysilane, APTMS) or carboxylic acid (3-aminopropanoic acid, APA) groups, which were further derivatized with the required proteins for the bioassays.

RESULTS AND DISCUSSION

Monitoring the Adsorption of Thiol Containing Molecules. The SNOPS strip was first tested for the detection of a long chain thiol. Figure 1 shows the response of the LSPR shift of an optimized strip with the concentration of 11-mercaptoundecanoic acid (MUA). The SNOPS strip presented good response to the presence of MUA, in terms of changes in both the LSPR full-width at half-maximum (fwhm) (Figure S-2 in the Supporting Information) and peak position (Figure 1), until a plateau is reached after 500 μM of MUA. A linear response was observed in the limit of low MUA concentrations (between 500 nM and 6 μM), which is 1 order of magnitude lower than reported for Ag nanosensors fabricated by electron beam lithography.[18] It is known that the shape of the LSPR envelope depends on the degree of aggregation,[19] especially the fwhm. Thus, our results point toward strong morphological changes at the sensor strip surface upon the binding of MUA. These changes are related to the aggregation of the NPs driven by the MUA adsorption. The large changes in the optical properties of the strips due to aggregation contribute to the higher sensitivity for MUA detection. The color changes due to the MUA adsorption could be verified by visual inspection,

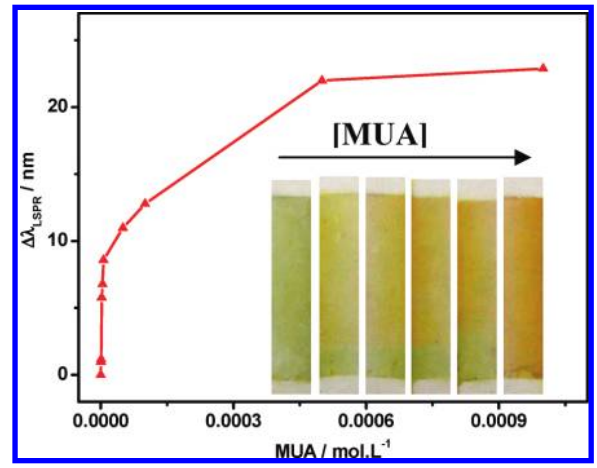


Figure 1. LSPR peak shift of the SNOPS strips with increasing MUA concentration in ethanol. The inset shows the image of the SNOPS strip after exposed to MUA at different concentrations (left to right: 0, 1 μM, 5 μM, 10 μM, 100 μM, and 10 mM). Images were taken in air.

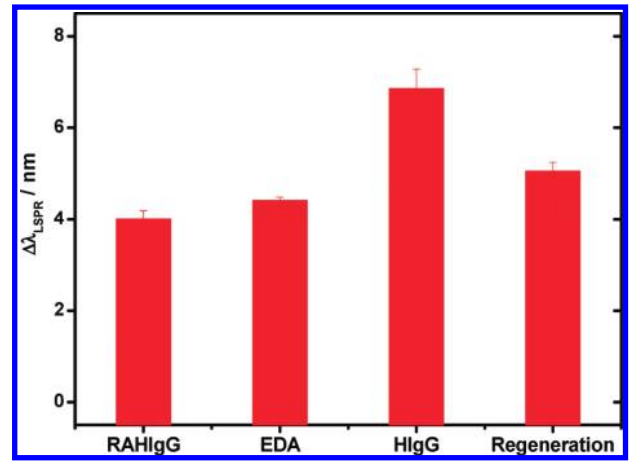


Figure 2. Shift of LSPR spectra of SNOPS strips after different stages of surface modification relative to unmodified Ag NPs.

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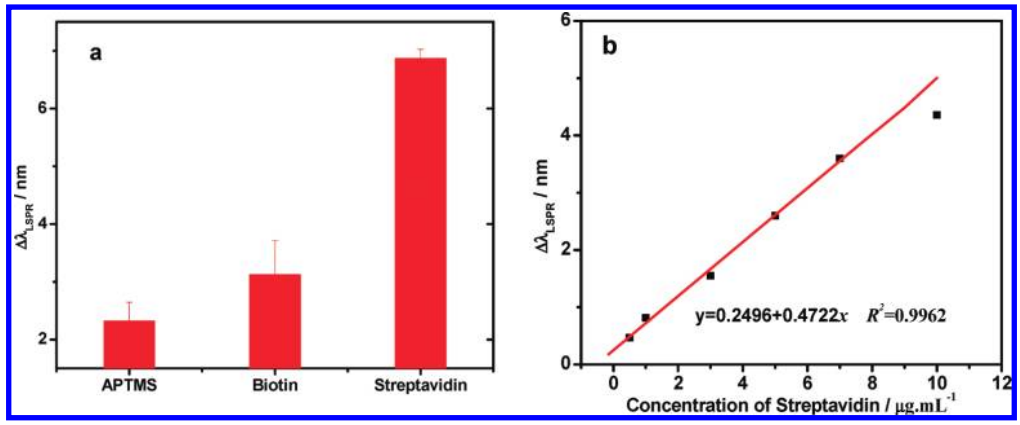

Figure 3. Monitoring the biotin−streptavidin binding: (a) LSPR peak shift after each surface modification step (relative to unmodified SNOPS) and (b) calibration curve for detection of streptavidin in solution at different concentrations. LOD (2σ) = 190 ng mL^{-1}.

as illustrated in the inset of Figure 1. This type of "detection" is akin to the color changes observed using "pH-papers" and might provide a new opportunity for the inexpensive SNOPS-based strips to be used as disposable biosensors.

Using SNOPS in Affinity Assays. The selectivity of a LSPR sensor is enabled by the immobilization of molecules on the metallic surface that target specific biological relevant species from solutions. The SNOPS strips were modified by different functional groups and proteins for biosensing, as shown in Scheme 1. As discussed above, however, the presence of strong binding functional group, such as thiols, induces the Ag NPs to aggregate. The SNOPS strips had to be carefully modified by the biomolecules to achieve a good degree of stability and reproducibility (about 4% RSD sample to sample) without strong aggregation.

The LSPR shifts after each step of surface modification in our model bioassay are shown in Figure 2. After incubation of *N*-hydroxysuccinimide (NHS)-activated SNOPS strips with the antigen RAHIgG for 15 min, a ~3.5 nm LSPR shift was observed. The next step was to expose the strips to an EDA solution to cap the unreactive NHS groups at the surface. This step caused a further LSPR shift of ~0.5 nm. The next step was the actual bioassay and involved the binding of the antibody HIgG onto RAHIgG. This affinity interaction provoked a further LSPR-shift of ~2.5 nm. The relative smaller shift for the adsorption of the second protein, HIgG, is expected, since the LSPR field penetration depth is about 20 nm and decays exponentially away from the surface.[2a,20,21] The sensor strip was regenerated when exposed to pH 3 buffer, and a blue shift in the LSPR (Figure 2) was observed due to the dissociation of the HIgG−RAHIgG complex.

Limit of Quantification Using the Streptavidin (SA)−Biotin Model System. The biotin−streptavidin scheme was used as a model system to determine the limit of quantification of the SNOPS sensor strips. Biotin was immobilized on the SNOPS strips using the APTMS sol−gel to produce an amino-functionalized surface (Scheme 1). The LSPR spectrum red-shifted following each step of the surface modification is presented in Figure 3a. The treatment of the SNOPS strip with APTMS sol−gel yielded a shift of ~2 nm. APTMS sol gel, similarly to MPTMS sol−gel,[17a,22] is a cross-linked silane "polymer" with large molecular weight. The binding of biotin further shifts the LSPR position by ~1 nm. Finally, after binding of SA (50 μg mL^{-1}), an additional shift of ~4 nm was observed (Figure 3a). Figure 3b presents a calibration curve for the detection of SA in solution using the SNOPS strips. The sensor presented a linear response in the SA concentration range between 9.5 and 189.4 nM, and the limit of determination (LOD) for SA was estimated as 3.5 nM (190 ng mL^{-1}).

CONCLUSIONS

A cheap, flexible, reproducible, and sensitive LSPR sensor platform was developed. This sensor platform consisted of PET films chemically modified with Ag NPs. The SNOPS strip is very sensitive to the adsorption of thiol containing molecules. Meanwhile, the SNOPS strips can also be used as a very versatile platform for monitoring protein−protein interactions and biotin−(strept)avidin binding by simply changing the surface functionality.

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SUPPORTING INFORMATION AVAILABLE

The experimental details, including optimization and calibration of the SNOPS. This material is available free of charge via the Internet at http://pubs.acs.org.

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