Supporting information for

Silver nanoparticles on a plastic platform for localized SPR bio-sensing

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The supporting material presented here contains the experimental section, the characterization and optimization of the silver nanoparticles on plastic sensor (SNOPS) strip, the analytical performance when exposed to bulk refractive indices change (calibration), and results for experiments with model proteins.

Supporting text:

Materials. Unless otherwise mentioned, ACS grade chemicals were used. $AgNO_3$, sodium citrate dihydrate, glucose, 3-aminospropyltrimethoxysilane (APTMS), 11-Mercaptoundecanoic acid (MUA), 1,2-ethylenediamine (EDA), 3-aminopropionic acid (APA), N-hydroxysuccinimide (NHS), N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), (+)-biotin N-hydroxysuccinimide ester (Biotin), Streptavidin (SA) from *Streptomyces avidinii*, human IgG (HIgG, lambda light chain from human mycloma plasma), Anti-human lambda light chain antibody from rabbit (RAH-IgG), and Acetylcholinesterase (AchE) from Electrophorus electricus are all from Sigma-Aldrich. PET films were the regular overhead transparency from BASICS office products LTD, Cambridge, Ontario. Before use, the PET sheets were cut into 9×40 mm strips. Ultrapure water with a resistivity of 18.2 MΩ.cm (from Barnstead NANOpure Diamond water purification system) was used in all experiments.

Absorbance measurements. A Cary 50 UV-vis spectrometer was used to record the absorption spectrum. The SNOPS strips were immersed in a quartz cuvette filled with glucose solutions of different concentrations to measure the sensor response to bulk RI changes (the RIs were measured using Pocket Refractiometer (ATAGO CO., Ltd). Water was used for all the remaining measurements.

Synthesis of Ag NPs and MPTMS sol-gel. The preparation of Ag NPs followed the procedure reported in the literature.[1] Briefly, a 500 mL $AgNO_3$ solution (5.29×10^{-4} M) was brought to boiling under vigorous stirring. Then, 10 mL of 1% sodium citrate solution was quickly added. The heating and stirring were kept for an hour and then only with stirring until the solution cool to room temperature. The as prepared Ag NPs showed an absorption peak at ~399

nm. The APTMS sol-gel preparation was following a sol-gel preparation method for 3-mercaptopropyltrimethoxysilane.[2] Briefly, 400 μL of APTMS and 332 μL of 0.1M HCl were added into 33 mL of water. The solution was vigorously stirred for at least 1 hour. The sol-gel solution shall be used the same day.

SNOPS strip fabrication (Scheme 1, text). The PET film was cut into 9×40 mm strips, sonicated in MeOH for 5min, and then mounted onto a custom made Teflon holder after dried with flowing N_2. Then, the strips were left in a plasma oven (Harrick Scientific Corp, Ossinng, New York) at high radio frequency for 20 min to activate the surface. Later, the strips were immersed overnight into EDA methanolic solution. After that, the strips were removed from the solution and sonicated in methanol and water for 3 min, respectively. Finally, the strips were rinsed with copious amount of water, and then soaked in Ag NPs solution for a desired amount of time. The Ag NPs modified PET strips then were rinsed with water to eliminate loosely bound Ag NPs and stored in water for future use. The SNOPS strips stored in this way were good for at least 3 weeks.

Optimization of the Ag NPs immobilization on PET. The plasma activated PET film was incubated overnight in different concentrations of methanolic solution of EDA before being immersed overnight in a Ag NPs dispersion (see Scheme 1, text). The effect of 1,2-ethylenediamine (EDA) concentration on the load of Ag NPs on PET surfaces is shown in Fig. S-1a. The absorbance significantly changed up to 30% (V:V) of EDA, and it stabilized after that. The error bars in Fig. S-1a represent the sample-to-sample (N=5) reproducibility and this relative standard deviation (RSD) in absorbance is shown to be less than 10% after the concentration of EDA reaches 30% (V:V). Considering these results, 40% (V:V) methanolic EDA solution were used in all subsequent experiments. The effect of the immersion time of Ag NPs deposition was

also explored and it is illustrated in Figure S-1b. The absorbance of the film after just 2 hours of incubation in Ag NPs solution was about 0.4, indicating that a significant amount of Ag NPs were deposited. The amount of Ag NPs immobilized increased slightly up to 6 hours of incubation, where an absorption plateau was reached. Interestingly, the relative sample-to-sample standard deviation decreases with the incubation time. The position of the LSPR peak is also plotted in Figure S-1b. The blue shift of the LSPR maximum wavelength (λ_{max}) with the Ag NPs deposition time is not unusual. Previous reports[4] have assigned this behavior to adsorbed electron donating species which increase the electron density of the MNPs.[4a] The Ag NPs strongly interacts with the amine modified PET film leading to the blue shift in the LSPR peak observed in Figure S-1b. The LSPR maximum wavelength stabilized after 6 hours, similarly to the observation for the absorption.

The effect of the Ag NPs incubation time on the sensitivity of the SNOPS strip to bulk refractive index (RI) changes was also examined. The results of the sensitivity to LSPR wavelength shifts are listed in Table S-1. A sensitivity of ~150 nm/RIU (refractive index unit), independent of the deposition time, was obtained. This level of sensitivity is comparable to earlier literature reports.[5] The results from Figure S-1 and Table S-1 suggest that a minimum of 4 hours of incubation in Ag NPs dispersion is required to achieve a reasonable sample-to-sample RSD% of the LSPR wavelength shifts. All the chemical and biochemical sensing experiments reported here were performed using SNOPS strips prepared with 40% (V:V) EDA methanolic solutions and 6-hours of incubation in Ag NPs.

Surface chemical modification and binding test (Scheme 1, in the main paper). In order to obtain carboxyl functionality on the surface, the SNOPS strips were immersed in 2 mM APA aqueous solution for 5 hours. After rinsing with water, the carboxyl group was activated through

EDC/NHS procedure (2 hours) before test its capability of binding proteins. Two experiments were carried out after this step. We firstly tested the reproducibility of the SNOPS strips for monitoring the protein adsorption. As shown in Scheme S-1, the EDC/NHS activated sensor strips were incubated in AchE (50 $\mu g.mL^{-1}$) PBS buffer solution overnight. Then they were rinsed thoroughly with PBS buffer before absorption measurements. The second experiment is to examine the protein-protein interaction. Firstly, the EDC/NHS activated strips were incubated in RAH-IgG (40 $\mu g.mL^{-1}$) PBS buffer solution for 15 min. The un-reacted carboxyl group was blocked by the using of 2 mM EDA solution (20 min). Finally, the SNOPS strips were left in HIgG (50 $\mu g.mL^{-1}$) PBS buffer solution for another 40 min. PBS buffer rinse was performed between steps before recording absorption spectra. A pH 3 solution contains HCl and NaAc was used to rinse off the bound HIgG as a double check for the binding.

In order to modify the Ag NPs surface with amine functionality, the SNOPS strips were left in APTMS sol gel for 20 min (Scheme 1, text). After rinsing with water then PBS buffer, the amine activated sensor strips were incubated in Biotin DMSO solution (4 $mg.mL^{-1}$) for 1 hour followed by rinsing with water.[3] To bind SA, the strips were left in SA PBS buffer solution for 30 min. After rinse with PBS buffer, the SA bound SNOPS strips were stored in PBS buffer again before absorption measurement.

Monitoring protein adsorption: stability and reproducibility. The surface modification procedure, described in scheme S-1, was tested using a model protein, Acetylcholinesterase (AchE), on multiple SNOPS strips. The sequential steps leading to the protein immobilization was followed by measuring the LSPR peak shifts of the strips and the results are shown in Fig. S-3. The LSPR spectra shift following the adsorption of 3-aminopropionic acid (APA) was rather small (~0.5 nm), despite the fact that this species binds directly to the surface of the Ag NPs, in a

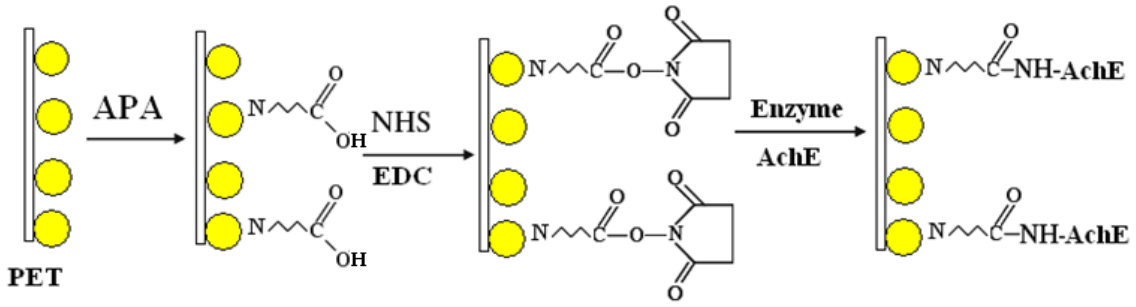
region where the local electromagnetic field is expected to be strongest.[5a, 6] This small shift can be rationalized by taking into account the size of the molecule and the binding group. APA is a relatively small molecule (MW = 89 a.m.u.) and the perturbation of local RI due to its adsorption is expected to also be small. Another contribution to the small shift is that APA binds to the silver surface through its amino group. It is known that amine is an electron donating specie and the adsorption of amino-containing species to MNPs can cause the LSPR to blue shift.[4] This effect was perfectly corroborated in the next step of surface modification, the coupling of N-hydroxysuccinimide (NHS), which led to a further ~ 2.5 nm redshift, despite the fact that this molecule would be away from the surface of the Ag NPs (hence experiencing weaker SP field than APA) and has comparable size (MW = 115 a.m.u.) to APA. The largest LSPR peak shift observed in Fig. S-3 is caused by the adsorption of protein AchE. A shift of ~6.5 nm (after APA modification) was observed (notice that the binding of the protein replaces the NHS group, see Scheme S-1) in this case. More importantly, a percent relative standard deviation (RSD%) of only 4% was observed by considering the LSPR spectrum shifts from all the different SNOPS strips measured (5 samples), indicating a good degree of sample-to-sample reproducibility for the SNOPS strips.

Supporting Figures, Schemes, and tables

Table S-1. Sensitivities of SNOPS strips fabricated with different incubation times in Ag NPs dispersion.*

Ag NPs incubation time	Sensitivity	R^2
2-hour	146.6	0.9956
4-hour	146.1	0.9852
6-hour	135.3	0.9939
8-hour	154.3	0.9964
10-hour	152.5	0.9933

*The LSPR wavelength was measured for strips immersed in glucose solutions of different concentrations. Fitting equation: $y = Bx + A$, where x is the RI, y is the LSPR peak position, and B is the sensitivity. The sensitivities are in nm/RIU (bulk).



Scheme S-1. Surface modification steps for protein immobilization on a SNOPS strip.

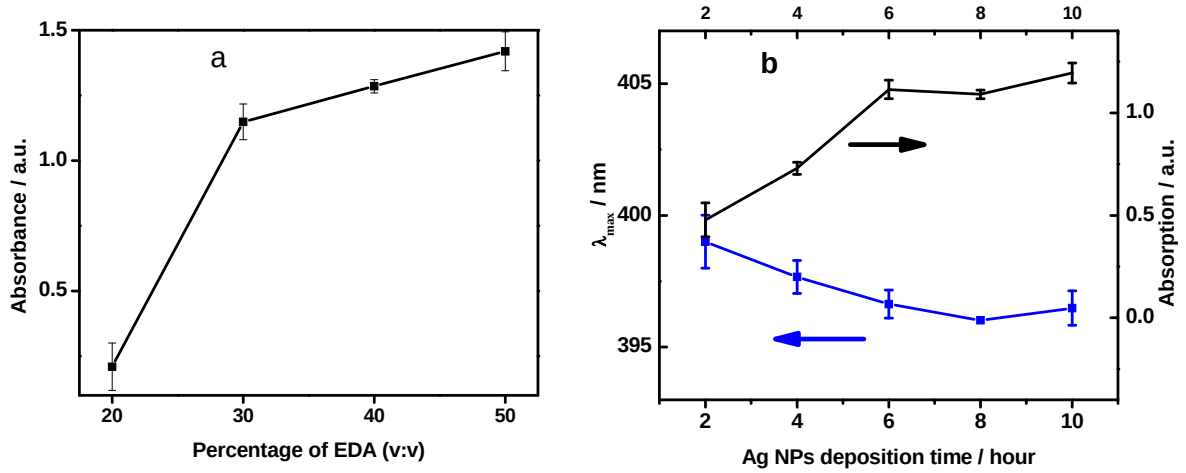


Figure S-1: Optimization of the Ag NPs immobilization on PET strips. a) Effect of the EDA concentration; b) effect of the incubation time in Ag NPs solution (with 40% of EDA in methanol (V:V)). The error bars are the sample-to-sample standard deviation (N=5). The lines in the graphs are only connecting the experimental points.

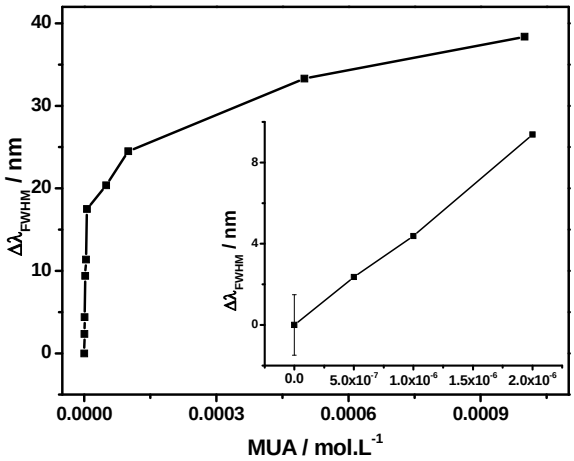


Figure S-2: Changes in FWHM of the LSPR peak of the SNOPS strip in the presence of MUA. The strips were incubated in MUA solutions of various concentrations for 2 hours before measurements. Inset shows the zoom-in of the FWHM change at low MUA concentration. The zero MUA concentration in the inset was obtained by incubate 3 SNOPS strips in pure ethanol for 2 hours.

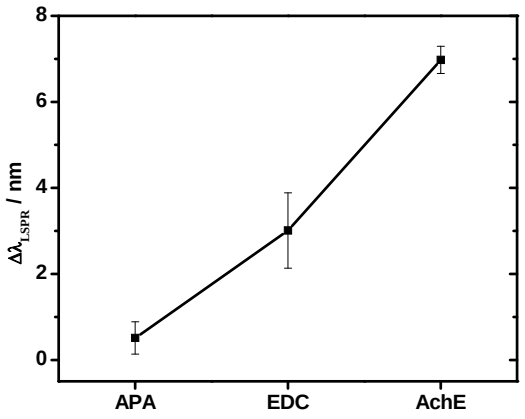


Figure S-3: LSPR peak shift due to surface modifications (relative to unmodified Ag NPs). Error bar shows the sample-to-sample standard deviation from 5 SNOPS strips.

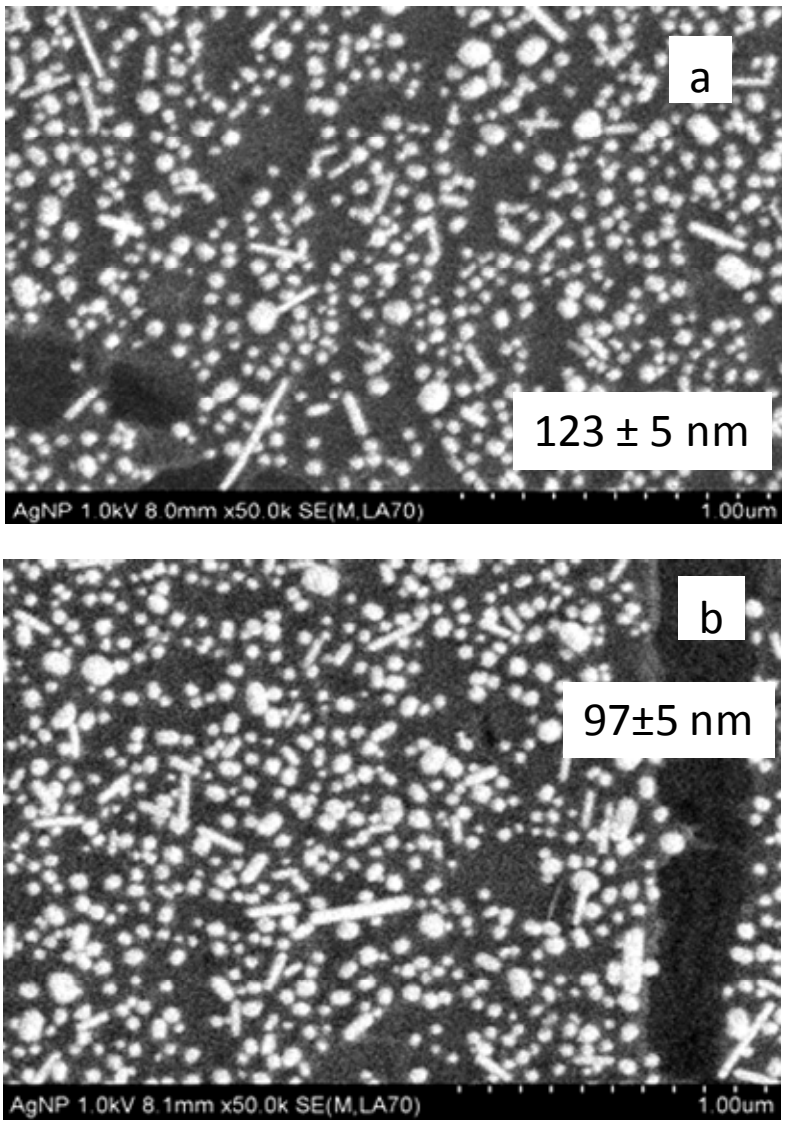


Figure S-4: Representative SEM images of the SNOPS strip before (a) and after (b) exposing to MUA solution. The average "surrounding distance" (distance from centre particle to surrounding particles), measured by randomly picking up groups (>20 groups) of Ag NPs, is indicated in each image. It was found that there is significant change (decrease of surrounding distance) after exposing to MUA. Note that the quality of the image is limited due to the charging effects. The cracks shown on both images are from the PET plastic.

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