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Silica-Stabilized Gold Island Films for Transmission Localized Surface Plasmon Sensing

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Abstract: Ultrathin gold films prepared by evaporation of sub-percolation layers (typically up to 10 nm nominal thickness) onto transparent substrates form arrays of well-defined metal islands. Such films display a characteristic surface plasmon (SP) absorption band, conveniently measured by transmission spectroscopy. The SP band intensity and position are sensitive to the film morphology (island shape and inter-island separation) and the effective dielectric constant of the surrounding medium. The latter has been exploited for chemical and biological sensing in the transmission localized surface plasmon resonance (T-LSPR) mode. A major concern in the development of T-LSPR sensors based on Au island films is instability, manifested as change in the SP absorbance following immersion in organic solvents and aqueous solutions. The latter may present a problem in the use of Au island-based transducers for biological sensing, usually carried out in aqueous media. Here, we describe a facile method for stabilizing Au island films while maintaining a high sensitivity of the SP absorbance to analyte binding. Stabilization is achieved by coating the Au islands with an ultrathin silica layer, ca. 1.5 nm thick, deposited by a sol−gel procedure on an intermediate mercaptosilane monolayer. The silica coating is prepared using a modified literature procedure, where a change in the reaction conditions from room temperature to 90 °C shortened the deposition time from days to hours. The system was characterized by UV−vis spectroscopy, ellipsometry, XPS, HRSEM, AFM, and cyclic voltammetry. The ultrathin silica coating stabilizes the optical properties of the Au island films toward immersion in water, phosphate buffer saline (PBS), and various organic solvents, thus providing proper conditions where the optical response is sensitive only to changes in the effective dielectric constant of the immediate environment. The silica layer is thin enough to afford high T-LSPR sensitivity, while the hydroxyl groups on its surface enable chemical modification for binding of receptor molecules. The use of silica-encapsulated Au island films as a stable and effective platform for T-LSPR sensing is demonstrated.

Introduction

Metal nanoparticles (NPs) exhibit unique optical properties deriving from collective oscillation of conduction electrons upon interaction with electromagnetic radiation, the so-called localized surface plasmon (SP) resonance.[1] The SP absorption band shape, amplitude, and frequency of maximum absorbance of an ensemble of isolated metal NPs depend on the effective dielectric constant of the surrounding medium, as well as on geometrical factors, that is, NP shape and size distribution.[2−4] The localized SP coupling observed in dense assemblies of NPs, either in solution or immobilized on solid substrates, introduces an additional factor, which strongly influences the conditions of the SP resonance.[5−7] Sensors based on localized SP resonance can therefore be divided into two broad groups, sensors based on monitoring changes in the dielectric constant of the immediate environment[3,8−14] and sensors based on changes in SP coupling.[15−18] A combination of these effects was also exploited in localized SP sensing.[19,20]

Changes in the localized SP band of discontinuous Au or Ag films upon analyte binding can be conveniently monitored

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using standard spectrophotometric equipment in the transmission mode, presenting a notable advantage in sensing applications. This method of transduction was termed transmission localized surface plasmon resonance (T-LSPR) spectroscopy.[21−24] Development of a reliable platform for T-LSPR sensing requires nanoparticulate metal films showing stable and reproducible optical properties. Change in the optical properties of metal island films resulting from morphological changes occurring upon exposure to solvents and analytes introduces uncertainty in the detection. This issue is tackled here by designing a scheme for stabilizing evaporated Au island films.

The structural and optical properties of Au and Ag nanoisland films prepared by evaporation onto transparent substrates have been studied in two basic configurations, that is, ordered nanoparticle arrays prepared by nanosphere lithography[7,12] and random island films prepared by direct evaporation.[10,11,24,25] In the latter case, we have shown that ultrathin (typically $\leq$10 nm nominal thickness) Au island films vapor-deposited onto transparent substrates display a SP extinction peak at 550−800 nm, whose shape, intensity, and position depend on the island morphology, determined by the evaporation conditions and postdeposition treatment. Changes in the SP extinction band (intensity and wavelength of maximum absorbance) resulting from binding of various molecules to the Au islands were measured in the transmission mode.[10,11,24,26] A linear relationship was shown between the surface coverage by adsorbing molecules, bound either directly to the Au or through a receptor layer, and the change in the SP intensity and wavelength,[10,11,24] crucial for quantitative sensing. T-LSPR spectroscopy was shown to be widely applicable, with a sensitivity that depends on the film preparation conditions[27] and under optimized conditions is comparable to that of SPR sensing.[24] The major advantage of T-LSPR sensing over conventional SPR sensing lies in the low-cost samples and simple experimental setup, which can be reduced to measurements at a single wavelength in a transmission configuration. This points to promising possibilities in disposable sensor kits for home, clinic, or field applications.

Preparation of noble metal (Au, Ag) island films with a stable optical response presents a challenging task. The substantial mobility of metal islands, especially Au, on common substrates (glass, transparent oxides) causes changes in the SP coupling and influences the spectrum shape. We have addressed this problem by evaporation of Au on mercaptosilane-modified glass substrates[28] followed by mild annealing.[25] The latter allows optimization of the optical response without substantially compromising the island adhesion to the glass. Despite the improved adhesion, the optical response of the Au island films remains sensitive to immersion in solvents.[25,29]

The sensitivity of the optical properties of Au and Ag island films to interaction with solvents is well-known.[30,31] It is assumed that changes in the optical response result from structural instability of the islands in solvents, reflecting an interplay between the island surface energy and interaction with the substrate. Although this problem can sometimes be overcome by stabilization of the optical response in background solution before the sensing step,[11,12,26] a more general approach to stabilization of the morphology and optical response of Au island films is required for the development of T-LSPR-based transducers.

Ultrathin films of silica present robust and transparent layers used for coating of continuous[32−34] and island-type[35−39] metal films as well as metal NPs.[40,41] Several approaches have been used for silica film deposition: vacuum evaporation,[35,38] spin-coating of silica sol,[33,36,37,42] and sol−gel formation in stagnant solution.[32,39−41] Good adhesion of sol−gel silica layers to Au or Ag was achieved using self-assembled monolayers (SAMs) of mercapto- or aminosilanes as a coupling layer.[36,37,39−41,43,44] In this approach, the coupling silane SAM introduces silanol groups on the NP surface, providing chemical affinity for silica, similar to that of oxide surfaces. The thickness of silica coatings prepared by the different techniques varied between ca. 1.0 and 10 nm. The thinnest films were reported by Liz-Marzán and co-workers for silica coating on Au NPs.[40,41]

In the present work, the optical properties of Au island films prepared by evaporation onto silanized glass substrates were stabilized toward immersion in solvents by coating the islands with an ultrathin sol−gel derived silica film. The limited distance sensitivity of T-LSPR measurements (a few tens of nanometers in optimized systems[27]), combined with the relatively thick recognition interfaces applied in biosensing, require minimization of the silica film thickness to preserve transducer sensitivity. The two-step method presented here includes self-assembly of a monolayer of 3-mercaptopropyl trimethoxysilane (MPTS) on the Au islands, followed by deposition of an ultrathin (ca. 1.5 nm) silica layer from a sodium silicate solution adjusted to a pH low enough to promote polymerization of the silicate groups. Carrying out the deposition reaction at 90 °C rather than at room temperature as in the published procedure[41] shortens the time necessary for formation of the silica shell from days to hours, without affecting the coating quality. Growth of the silica layer

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was monitored by UV−vis spectroscopy, ellipsometry, and XPS measurements, while the surface morphology and blocking properties of the layer were characterized by high-resolution scanning electron microscopy (HRSEM), atomic force microscopy (AFM), and cyclic voltammetry (CV). Use of silica-coated Au island films as stable and effective T-LSPR transducers is demonstrated by monitoring changes in the optical response upon binding of protoporphyrin IX and Ag NPs to a receptor layer on the silica surface.

Experimental Section

Chemicals. Sodium silicate solution (27 w/%), 3-mercaptopropyl trimethoxysilane (MPTS), 3-aminopropyl trimethoxysilane (APTS), protoporphyrin IX (PPIX), and $Ru(NH_3)_6Cl_3$ were purchased from Aldrich. Methanol and ethanol, absolute, analytical grade, were purchased from J.T. Baker. The ion-exchanger Amberlite IR-120 was purchased from Merck. $K_3Fe(CN)_6$, analytical, was purchased from BDH chemicals. Phosphate buffer saline (PBS) solution was prepared by 10-fold dilution of commercial reagent GIBCO D-PBS(10X)-$CaCl_2$, -$MgCl_2$ (Invitrogen) with triply distilled water.

UV−Vis Spectroscopy. Extinction spectra at normal incidence were measured in air using a Varian Cary 50 UV/vis spectrophotometer. The wavelength scan range was 350−1000 nm, and the scan speed was 600 nm min^{-1}. The samples were placed in a special holder enabling transmission measurements of the same spot on the slide during all experimental stages.

Cyclic Voltammetry (CV). CV was carried out with two redox probes: $Fe(CN)_6^{3-}$ (1 mM) and $Ru(NH_3)_6^{3+}$ (0.5 mM) in 0.1 M sodium sulfate solution. Experiments were performed in a three-electrode cell with a K_2SO_4-saturated Hg/Hg_2SO_4 reference electrode and a Pt counter electrode. Before each experiment, the solution was deaerated by bubbling high-purity N_2, while during the CV an inert environment was maintained by passing N_2 above the solution. The CV always started at the most positive potential, and the scan rate was 0.05 V s^{-1}. The electrochemical measurements were carried out using (bare or silica-coated) 20-nm-thick, continuous Au films on silanized glass slides. The slides were partially immersed in the electrolyte solution; after completion of the experiment and emersion from the solution, the electrode area (ca. 0.5 cm^2) was determined using the clearly seen wetting line.

Ellipsometry. Ellipsometric measurements were carried out with a Rudolph Research Auto-EL IV null ellipsometer, at an angle of incidence $\phi = 70°$ and a wavelength $\lambda = 632.8$ nm. The same four points were measured on each sample before and after film formation.

Atomic Force Microscopy (AFM). AFM images were recorded in air using a PicoSPM instrument (Molecular Imaging, USA) operated in the acoustic AC (AAC) mode. The cantilevers used were NSC12 series of ultrasharp silicon (MikroMasch, Estonia), with a resonant frequency of 100−200 kHz and an average tip radius of $\leq$10 nm.

X-ray Photoelectron Spectroscopy (XPS). XPS measurements were carried out using a Kratos Axis-HS XPS system, with a monochromatized Al Kα X-ray source (1486.6 eV). For Si high-resolution analysis, the Si 2s was chosen rather than the Si 2p region due to interference of the latter with the intense Au 4f peak.

High-Resolution Scanning Electron Microscopy (HRSEM). Images were obtained using a JSM-6700F high-resolution scanning electron microscope with a cold field emission electron source and an upper built-in SE detector. Low-energy SE signal suppression was performed to reduce the charging effect in the SE images.

Gold Film Preparation. Glass substrates for Au evaporation were cut from glass microscope cover slides (Deckglaser No. 2) into 18 × 9 mm pieces and cleaned using one of the following procedures: (i) The glass slides were immersed twice in fresh “piranha” solution (1:2 H_2O_2:H_2SO_4) for 20 min, followed by washing three times in triply distilled water and three times in methanol. (ii) The slides were immersed in fresh “piranha” solution for 1 h, followed by treatment in a hot (70 °C) solution of 1:1:5 H_2O_2:NH_4OH:H_2O for 1 h and extensive rinsing in methanol. (*Caution: Pirahna solution reacts violently with organic materials and should be handled with extreme care*.) The two pretreatment procedures produced similar results. After being cleaned, the glass slides were modified with APTS by overnight immersion in a 10 v/% APTS solution in methanol. The silanized glass slides were sonicated three times in methanol, washed with ethanol and dried under a stream of nitrogen, and then transferred to the evaporation chamber. Silicon wafers were cut to 20 × 10 mm slides, immersed twice in fresh “piranha” solution for 20 min, immersed in a hot (70 °C) solution of 1:1:5 H_2O_2:NH_4OH:H_2O for 20 min, and washed three times in triply distilled water and three times in methanol. The Si/SiO_2 slides were then left in a 10 v/% APTS solution in methanol for 3 h. The silanized silicon slides were sonicated three times in methanol and washed with ethanol, dried under a stream of nitrogen, and mounted in the evaporation chamber. Au evaporation on glass or silicon substrates was carried out in a cryo-HV evaporator (Key High Vacuum) equipped with a Maxtek TM-100 thickness monitor. Homogeneous deposition was achieved by moderate rotation of the substrate plate. Au (99.99%, Holland-Moran, Israel) was evaporated from a tungsten boat at 2−4 μTorr. For preparation of Au island films on glass, a low deposition rate of 0.01 nm s^{-1} was applied.[10,11,24,25] Continuous, 20-nm-thick Au films on glass and 100-nm-thick Au films on Si/SiO_2 were deposited at a rate of 0.1 nm s^{-1}.[45] Postdeposition annealing of Au-covered slides was carried out in air at 200 °C for 20 h using a Ney Vulcan 3-550 furnace. The heating rate was 5 °C min^{-1}, and the annealed slides were left to cool in air to room temperature. The annealing temperature was chosen to maintain good adhesion of the Au to the substrates, provided by the organic silane monolayer.[25,27] The continuous Au films (20 nm on glass, 100 nm on silicon) provide smooth, (111) textured surfaces suitable for common characterization techniques (ellipsometry, electrochemistry, XPS, etc.).[45] The reproducibility of the Au evaporation was evaluated by measuring the SP extinction spectra of a batch of 24 evaporated samples, comprising 5 nm Au evaporated on APTS-treated glass and annealed. The variability (standard deviation) in the wavelength (λ_{max}) and maximum extinction (E_{max}) of the SP band is typically 0.4 nm and 0.002 abs. units, respectively.

Formation of Silica Coatings. The thin (ca. 1.5 nm) silica layers used for stabilizing the optical response of Au island films were deposited by generally following the method described by Liz-Marzán et al.[41] with certain modifications. The general scheme of silica layer deposition is presented in Figure 1. Au-coated glass slides, annealed, were washed in ethanol for 20 min and dried under a stream of nitrogen. The slides were then immersed for 1 h in a freshly prepared solution of 2 mM MPTS in ethanol, washed for 20 min in ethanol, and dried under a stream of nitrogen, to obtain an MPTS primer monolayer on the Au.

Sodium silicate solution (~1.5 w/% SiO_2, pH $\approx$ 12) was prepared by dilution of 2 mL of the original solution (27 w/% SiO_2) with triply distilled water to a final volume of 50 mL. The strongly acidic cation exchanger Amberlite IR-120 was used for further adjustment of the solution pH to 8.5−9. For silica deposition, the slides were then dipped for a certain time period in a 1.5% sodium silicate solution kept at a predetermined constant temperature. After removal from the sodium silicate solution, the silica-coated Au substrates were washed with water and dried under a stream of nitrogen. Transmission UV−vis spectra were measured in each step of the transducer preparation.

In the case of continuous Au films (20 or 100 nm thick), the silica coating procedure was preceded by 10 min UV-ozone treatment, 20 min ethanol dip, and drying under a stream of nitrogen.[46] Formation of silica coatings on continuous Au films was monitored by ex-situ ellipsometry. The ellipsometric thicknesses were calculated using a film

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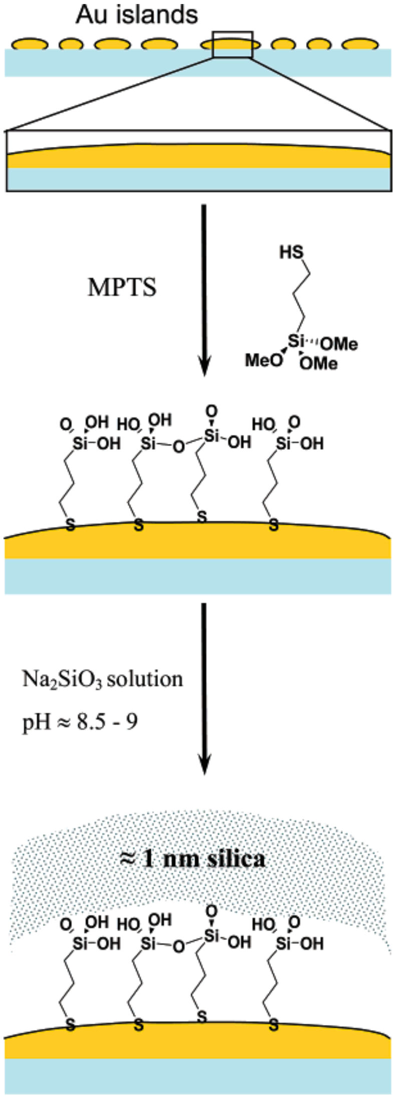

Figure 1. Schematic presentation of the stepwise procedure for preparation of ultrathin silica layers on Au island films. (a) Gold islands on silanized glass. (b) Formation of an MPTS SAM on the gold surface. (c) Deposition of an ultrathin sol−gel silica film. Sizes of features in the scheme are not to scale.

refractive index of $n_f = 1.5$, $k_f = 0$, based on the refractive index of silica layers reported by Philipse et al.[47] ($n_f = 1.456$).

Stability Tests. Stability tests were carried out on 5 nm Au island films coated with silica (prepared at 90 °C for 2 h). The stability of the Au plasmon peak was tested by immersion in four different liquids: ethanol, methanol, water, and PBS. Spectra were measured in air, before and after dipping.[48] After the initial transmission UV−vis spectrum was obtained, the sample was dipped in a given liquid for 20 min, removed, and dried under a stream of nitrogen. In the case of PBS, after 20 min immersion the sample was washed with water before drying with nitrogen. After drying, the final UV−vis spectrum was measured.

Binding of Protoporphyrin IX (PPIX) and Ag Nanoparticles (NPs). 5 nm Au island films coated with a silica layer (prepared at 90 °C for 2 h) were washed in methanol for 20 min, dried under a stream of nitrogen, and left overnight in a solution of 10 v/% APTS in methanol. The samples were washed three times in methanol, then washed in ethanol and dried under a stream of nitrogen. This procedure provides an amine-terminated silica surface capable of electrostatic binding of negatively charged species. For immobilization of the dicarboxylic acid porphyrin PPIX,[45] the amine-terminated samples were left in a saturated solution (0.2 mM) of PPIX in ethanol overnight, washed with ethanol, and dried with nitrogen. A spectrum of PPIX dissolved in ethanol was taken at 4 μM concentration. For Ag NP binding, silica-coated Au island films with an APTS overlayer were dipped overnight in a solution of citrate-stabilized Ag NPs, washed with water, and dried under a stream of nitrogen. Citrate-stabilized Ag NPs were prepared according to a literature procedure.[49]

Results and Discussion

Silica Coating on Thin, Continuous Au Films. Our basic objective was stabilization of Au island transducer films using ultrathin silica coatings. This required development of schemes for silica coating and film characterization on Au islands. The latter is complicated by the fact that most characterization techniques are difficult to perform on island films. We therefore chose to develop the basic coating scheme on thin, continuous Au films and then transfer the methodology to Au island films.

The growth of silica layers on continuous Au substrates (see Experimental Section) was followed by ex-situ ellipsometry. The kinetics of silica layer formation were monitored by measurement of the ellipsometric parameter Δ at different times of immersion in sodium silicate solution. The total thickness of the ovelayer comprising a silica film on a mercaptosilane monolayer was calculated assuming formation of a uniform layer with a refractive index $n_f = 1.5$, $k_f = 0$. In experiments performed at room temperature (23 °C), a limiting film thickness of ∼1.1 nm was reached after 72 h. Further growth was extremely slow, in general agreement with earlier observations,[40] where a 1.5 nm overlayer was reported after 7 days and 2.3 nm after 60 days of immersion.

To achieve faster deposition, silica layer formation was carried out at higher temperatures (40−90 °C), showing substantial acceleration of the kinetics. After 1 h of silica deposition at 90 °C under otherwise similar conditions, the thickness of the overlayer was ∼1.0 nm, while a silica layer thickness of 1.4−1.5 nm was reached after 1.5−2.0 h deposition. The latter immersion time (2 h) was chosen for the preparation of silica overlayers on Au island films in the present work. As shown below, the thickness of the silica layer formed on Au islands is similar to that measured ellipsometrically on continuous Au substrates. The silica layer on the Au islands is reminiscent of the robust, continuous silica layers of comparable thickness formed on Au NPs[40] and is expected to provide effective encapsulation.

The surface elemental composition of slides at different stages of silica coating on continuous Au films was probed by XPS. Figure 2 shows high-resolution scans in the Si 2s region for samples with an MPTS SAM and a silica layer grown at different temperatures. The Si 2s binding energy of 152.8−153 eV in the MPTS SAM coincides with the value measured for C−Si−O in a SAM on Au.[50] An increase in the area under the Si 2s peak and a shift to a higher binding energy are observed as the silica layer is formed. The energy shift reflects the deposition of SiO_2 (higher oxidation state) on the MPTS. For

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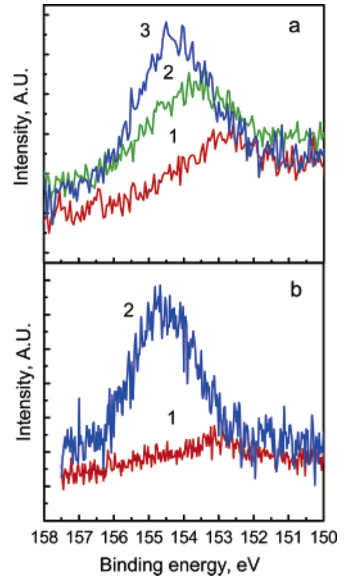

Figure 2. High-resolution XPS spectra for the Si 2s peak at different stages of silica coating formation. (a) Silica deposition at 23 °C on continuous, 100 nm Au film on silicon: (1) after formation of an MPTS SAM, (2) after 24 h in 1.5 w/% SiO_2 solution, and (3) after 4 days in 1.5 w/% SiO_2 solution. (b) Deposition of a silica layer at 90 °C on continuous, 20 nm Au film on silanized glass: (1) after formation of an MPTS SAM, and (2) after 2 h in 1.5 w/% SiO_2 solution.

samples prepared at 90 °C, the ratio O/Si is 3.0 after MPTS formation, decreasing to 2.53 ± 0.29 after silica coating (2 h). The former value is in agreement with the 3:1 stoichiometric O:Si ratio in MPTS, while the decrease in the O/Si ratio reflects the formation of a silica layer with a stoichiometric O:Si ratio of 2:1 on the MPTS SAM. A similar change in the O:Si ratio from 3.7 for Au/MPTS to 2.6 for Au/MPTS/silica (steady state) was observed for samples prepared at 23 °C. Hence, the XPS results are consistent with the formation of an ultrathin overlayer of SiO_2 upon immersion of Au/MPTS in sodium silicate solution.

Electrochemistry at Silica-Coated Au Electrodes. Sol−gel silica films are known to be nanoporous.[51] In the present work, cyclic voltammetry (CV) of the charged redox probes $Fe(CN)_6^{3-/4-}$ and $Ru(NH_3)_6^{3+/2+}$ in neutral ($pH \approx 7$) solution was used to characterize the blocking properties of silica layers deposited on continuous Au films. Comparison of CVs at bare and silica-coated Au electrodes (Figure 3) reveals distinct differences in the access of negative and positive electroactive ions to the underlying electrode. The electrochemical response of $Fe(CN)_6^{3-}$ at an Au electrode coated with a silica layer deposited at 90 °C shows complete blocking of the electrode reaction of the anionic probe (Figure 3a, line 2). Similar blocking properties were recently reported for a multilayer MPTS film on Au electrodes.[44] The cationic redox probe $Ru(NH_3)_6^{3+}$ (Figure 3b) shows a totally different behavior; that is, the CV at the silica-coated electrode is nearly identical to that at the bare Au electrode (the current is slightly enhanced, see below), exhibiting essentially free access of the cationic species to the electrode.

The permeability of ionic redox probes through silica layers is strongly influenced by electrostatic interactions. The point of zero charge (pzc) of sol−gel derived silica is around pH 2−4;[52] therefore, at the neutral pH applied here the silica layer is negatively charged. The negative silica surface charge furnishes an efficient electrostatic barrier to transport of the negatively charged $Fe(CN)_6^{3-}$ ions, hence the effective blocking observed in the CV (Figure 3a). On the other hand, the positively charged $Ru(NH_3)_6^{3+}$ ions experience free access through the nanoporous silica layer (Figure 3b). The small increase in the peak current for $Ru(NH_3)_6^{3+}$ is attributed to some accumulation of positively charged ions in the silica pores. This minor effect is not detected when the electrode is transferred to background solution. The electrochemical results provide a clear indication of the silica layer integrity and absence of physical defects (cracks, holes).

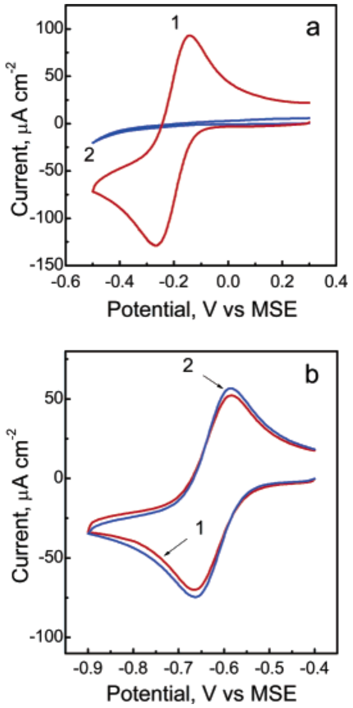

Figure 3. CV results for ionic species at bare and silica-coated Au electrodes (silica deposited at 90 °C for 2 h), first cycle is always shown. (a) CV of $Fe(CN)_6^{3-/4-}$ carried out in 1 mM $K_3Fe(CN)_6$ + 0.1 M Na_2SO_4: (1) bare Au, (2) Au/SiO_2. (b) CV of $Ru(NH_3)_6^{3+/2+}$ carried out in 0.5 mM $Ru(NH_3)_6Cl_3$ + 0.1 M Na_2SO_4: (1) bare Au, (2) Au/SiO_2.

Silica-Coated Au Island Films. As shown above, ultrathin (ca. 1.5 nm) silica layers grown at 90 °C on continuous Au substrates exhibit excellent integrity. Next, the same scheme was applied to the deposition of similar silica layers on discontinuous, Au island films. The substrates used were 5.0 nm (nominal thickness) Au island films, annealed. These substrates were previously shown by us to display superior distance sensitivity in T-LSPR spectroscopy.[27] Note that experiments with 2.5 nm (nominal thickness) Au island films (not shown here) produced similar spectroscopic results.

The morphology of silica coatings on 5.0 nm Au island films was studies by AFM and HRSEM (Figure 4). The Au islands in the AFM images appear larger in diameter, more rounded and closer to each other as compared to the corresponding HRSEM images, resulting from tip convolution. The combined AFM and HRSEM data provide the accurate 2D shape of the Au islands at different stages (HRSEM), together with viable

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(51) Nair, B. N.; Keizer, K.; Okubo, T.; Nakao, S. I. *Adv. Mater.* **1998**, *10*, 249.

(52) *Surfactant Science Series*; Papirer, E., Ed.; Marcel Dekker Inc.: New York, 2000; Vol. 90, p 753.

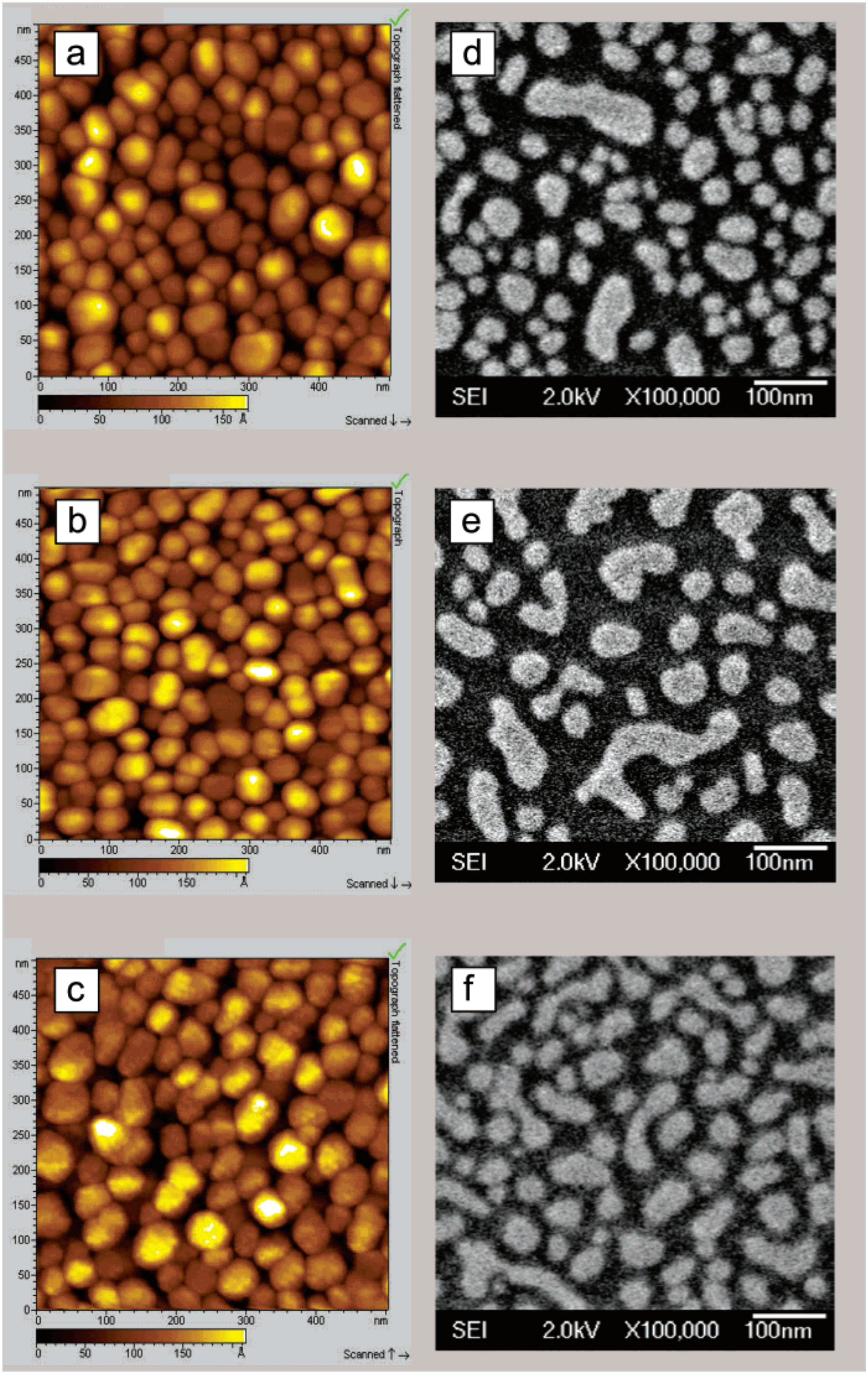

Figure 4. AFM (a−c) and HRSEM (d−f) images of 5 nm Au island films, annealed, at different stages of surface modification: (a,d) bare Au, (b,e) after assembly of an MPTS SAM, and (c,f) after silica deposition (at 90 °C, 2 h).

information on differences in the surface morphology effected by the silica coating (AFM).[25,53]

AFM images before and after MPTS adsorption are indistinguishable (Figure 4a,b), as expected for binding of a monolayer. Coating with the silica layer (90 °C, 2 h) is clearly seen as roughening of the surface (Figure 4c). The HRSEM images (Figure 4d−f) confirm that the 2D shape of the Au islands is not affected by the silica coating. No defects or cracks in the silica layer are seen on the length scale of Figure 4. This is in agreement with the macroscopic CV results, the latter showing compete blocking of anion penetration through the porous silica layer (Figure 3a).

Formation of a silica layer on Au island films was studied by T-LSPR spectroscopy, monitoring changes in the SP absorbance. Figure 5a shows UV−vis extinction spectra of a 5 nm Au island film, annealed, before (line 1) and after (line 2) MPTS adsorption, and after 2 h silica deposition at 90 °C (line 3). A change in the shape and intensity of the Au SP peak is seen after each step in the coating procedure, reflecting an increase in the effective refractive index of the Au islands' surrounding

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(53) Grabar, K. C.; Brown, K. R.; Keating, C. D.; Stranick, S. J.; Tang, S. L.; Natan, M. J. *Anal. Chem.* **1997**, *69*, 471.

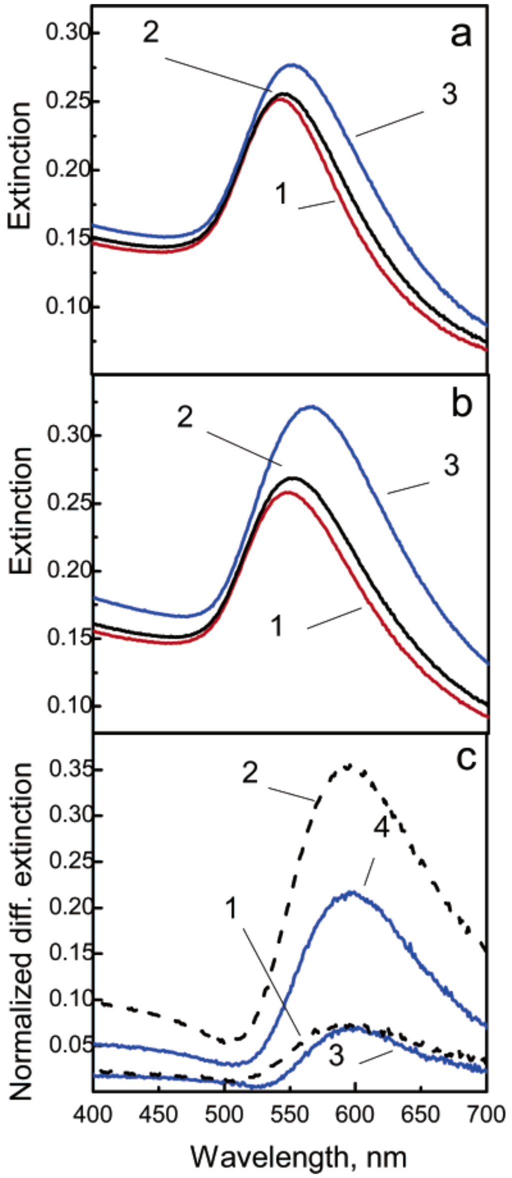

Figure 5. Extinction spectra recorded at different stages of preparation of multilayer structures on 5-nm-thick, annealed Au island films. (a) Silica coating: (1) bare Au; (2) after MPTS adsorption; and (3) after silica deposition (at 90 °C, 2 h). (b) Self-assembled coordination multilayer (from ref 54): (1) bare Au; (2) after SAM of bishydroxamate ligand; and (3) after binding of Zr(IV) ions followed by a layer of hexahydroxamate ligand. (c) Normalized differential spectra derived from the data in (a) and (b) (obtained by subtraction of the spectrum of the bare Au, normalized to the peak intensity of the bare Au): (1) Au/bishydroxamate SAM; (2) Au/bishydroxamate SAM+Zr(IV)+hexahydroxamate layer; (3) Au/MPTS SAM; and (4) Au/MPTS SAM+silica.

medium. The standard deviation of the differential intensity and the wavelength of maximum differential extinction are 1.2×10^{-3} abs. units and 0.9 nm, respectively, both within the accuracy of the spectrophotometric measurement. For different batches, the spread in the spectroscopic results is ca. ±0.01 abs. units and ±8.0 nm, respectively, reflecting a certain variability in the preparation conditions. The reproducibility is expected to improve in automated preparation of silica-stabilized T-LSPR transducers.

While the thickness of the silica layer on a continuous Au film was determined ellipsometrically (ca. 1.5 nm, see above), the same technique cannot be applied to the island film.

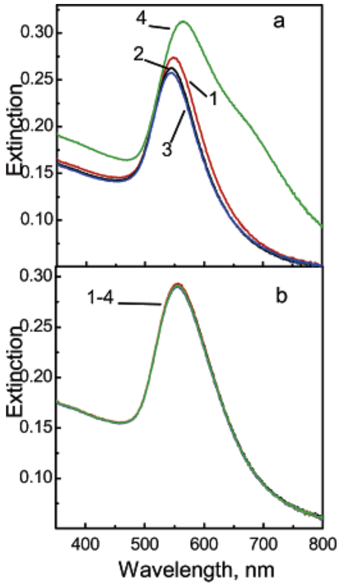

Figure 6. T-LSPR spectra (measured in air) of (a) bare Au island films (5 nm, annealed) and (b) coated with a silica layer (deposited at 90 °C, 2 h), before and after 20 min immersion in various liquids: (1) before immersion, (2) after EtOH or MeOH, (3) after water, and (4) after PBS.

However, the thickness of the silica layer on Au islands can be estimated using our published results on the distance dependence of T-LSPR spectra for the layer-by-layer preparation of coordination multilayers on Au island films.[27,54] Briefly, a bishydroxamate anchor SAM was prepared on Au, followed by binding of Zr^{4+} ions to which a layer of ligand molecules comprising multidentate (2 or 3) bishydroxamate functional groups is coordinatively bound. Additional sequential binding of Zr^{4+} ions and multidentate organic ligands led to regular growth of multilayer films on both continuous and island-type Au films. The multilayer thickness, which increases incrementally with added layers, was measured on continuous Au substrate by several methods (ellipsometry, transmission spectroscopy, AFM, XPS). For a metal−organic bilayer comprising Au/bishydroxamate SAM+Zr(IV)+a tridentate layer, a thickness of 1.9 nm was determined.[54] The refractive index of the multilayer, obtained by modeling of the optical data, was $n_f = 1.5$, $k_f = 0$.[54]

The morphology and optical properties (refractive index) of the silica and metal−organic multilayers on Au island films are similar; hence the thickness of the silica layer on the Au islands can be estimated from comparison of T-LSPR data for the two systems. T-LSPR spectra recorded after formation of a bishydroxamate SAM and after binding of Zr^{4+} ions and a layer of the tridendate (hexahydroxamate) organic ligand are shown in Figure 5b.[54] Differential spectra (after subtraction of the bare Au spectrum) for the preparation of the silica film and the organic bilayer are presented in Figure 5c. The difference spectra of the ligand monolayer (Figure 5c, line 1) and the MPTS monolayer (Figure 5c, line 3) are nearly identical, as expected. The spectrum of the silica layer (Figure 5c, line 4) lies between those of the organic monolayer (Figure 5c, line 1) and bilayer (Figure 5c, line 2). Based on the known thicknesses of the coordinated layers[54] and the linear dependence of the maximum differential extinction on the overlayer thickness,[27] the total

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[54] Wanunu, M.; Vaskevich, A.; Cohen, S. R.; Cohen, H.; Arad-Yellin, R.; Shanzer, A.; Rubinstein, I. *J. Am. Chem. Soc.* **2005**, *127*, 17877.

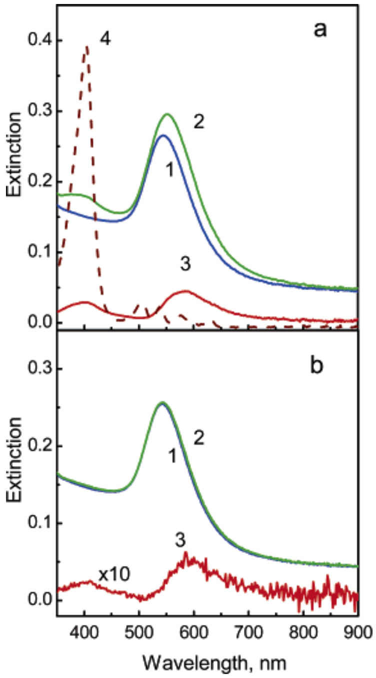

Figure 7. T-LSPR spectra (measured in air) for the binding of PPIX to silica-coated (90 °C, 2 h) Au island transducers. (a) Specific binding: (1) Au/silica+APTS; (2) after PPIX binding; (3) difference spectrum (2 − 1); and (4) spectrum of 4 μm PPIX solution in EtOH (optical path, 1 cm). (b) Nonspecific binding: (1) Au/silica; (2) after PPIX binding; and (3) difference spectrum (2 − 1, scaled ×10).

thickness of the MPTS+silica layer on the Au islands is estimated as ca. 1.2 nm. This value is in reasonable agreement with the thickness of ca. 1.5 nm measured ellipsometrically for the silica layer on a continuous Au film (see above).

Effect of Silica Coating on the Stability of T-LSPR Response. The stability of the Au SP absorbance toward immersion in various liquids (ethanol, methanol, water, PBS) was tested with and without a silica coating on Au island films. Measurements were carried out in air, before and after dipping. For bare Au island films (Figure 6a), the SP peak changes after immersion in each liquid. The most dramatic change is seen after dipping in PBS, the most common environment for biological sensing.

After coating of Au island films with a ca. 1.5 nm silica layer the stability of the optical response is dramatically improved (Figure 6b). UV−vis spectra of silica-coated Au island films measured before and after immersion in all of the liquids, including PBS, do not show any change in the SP absorbance within the accuracy of the measurement. This result establishes the feasibility of using silica-coated Au island films as transducers for chemical and biological sensing, ensuring that changes in the transmission spectra reflect primarily binding/release of target analytes.

Sensing with Silica-Coated T-LSPR Transducers. To demonstrate application of silica-stabilized Au island films as optical transducers in T-LSPR sensing, the system sensitivity to binding of analytes to receptor layers on the silica coating was tested. The sensitivity of T-LSPR response of Au island films to remote analyte binding was previously shown by us.[27] Distance sensitivity of >15 nm was obtained with 5 nm Au island films, annealed. The same kind of Au islands, coated with silica, was used in the present work. Two types of analytes were chosen to demonstrate T-LSPR sensing: the dicarboxylate porphyrin PPIX and citrate-stabilized Ag NPs.

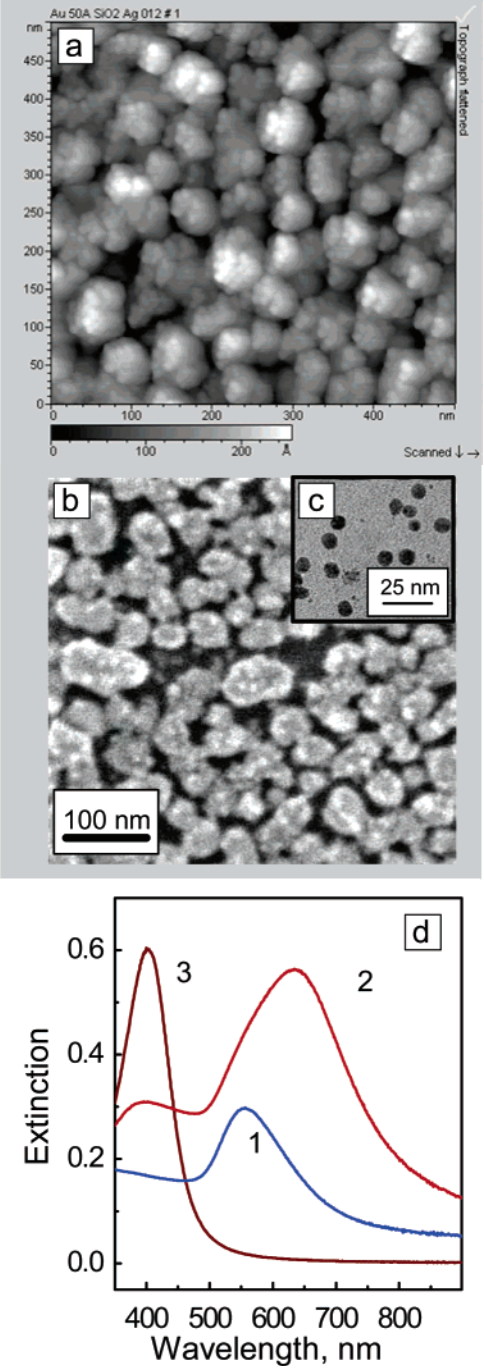

Figure 8. (a), (b) AFM and HRSEM images, respectively, of an Au/silica+APTS sample with a bound layer of Ag NPs. (c) TEM image of the citrate-stabilized Ag NPs. (d) Extinction spectra of: (1) Au/silica+APTS; (2) after binding of Ag NPs; and (3) spectrum of Ag NPs in solution (spectra 1 and 2 were measured in air).

PPIX has an intense absorption band with a maximum at 400 nm and a molar extinction coefficient $\epsilon \approx 10^5$ M^{-1} cm^{-1} (Figure 7a, line 4), allowing simultaneous monitoring of molecular absorption and changes in the Au SP band.[10,11] The SP absorbance of the Au island film around 550 nm is well separated from the PPIX molecular absorption band centered at 400 nm. For PPIX sensing, silica-coated Au island transducers were primed with a simulated receptor monolayer of the aminosilane APTS. Specific electrostatic binding of PPIX to APTS-modified samples was optically monitored, while unmodified samples (no APTS) were used as a nonspecific blank.

Figure 7a shows extinction spectra of an APTS-modified, silica-coated transducer before and after binding of PPIX. The appearance of PPIX molecular absorbance and the change in the Au SP band are clearly seen in the extinction spectrum after PPIX binding (Figure 7a, line 2) and particularly in the difference spectrum (Figure 7a, line 3). The latter also shows that the wavelength of maximum absorbance of silica-bound PPIX is very close to that measured in ethanolic solution. In a control experiment using an unmodified (receptor-free) transducer, the spectra before and after exposure to PPIX nearly coincide (Figure 7b, lines 1 and 2). Comparison of the differential spectra in Figure 7a (line 3) and Figure 7b (line 3, scaled ×10) reveals that the ratio between specific and nonspecific adsorption is approximately an order-of-magnitude in favor of specific electrostatic binding.

The PPIX sensing experiments show a submonolayer sensitivity of silica-coated T-LSPR transducers, consistent with our previous results that demonstrated nearly constant sensitivity of the T-LSPR response to analyte binding up to >15 nm from the Au island surface.[27] Quantification can be achieved by measuring changes in one (or more) of several spectral parameters, that is, maximum extinction intensity, wavelength of maximum extinction, or differential parameters.[11]

The sensitivity of the Au SP peak of silica-coated Au island transducers to changes in the dielectric properties of the immediate environment was also tested by electrostatic binding of Ag NPs: negatively charged, citrate-stabilized Ag NPs were bound to an APTS-modified silica overlayer on Au island film. Binding of the 9 nm (average diameter, see Figure 8c) Ag NPs to the silica coating was examined by AFM and HRSEM imaging. Figure 8a,b shows images of an APTS-modified silica-coated Au island film after Ag NP binding. The Ag NPs are clearly seen immobilized on the surface of the Au islands in the AFM (as protrusions) and HRSEM (as bright spots) images. The limited resolution of the AFM and HRSEM precludes a definite conclusion regarding possible aggregation of some Ag NPs during sample drying. A small number of NPs are seen in the area between Au islands in the HRSEM image.

Figure 8d shows changes in the extinction spectrum of a silica-coated Au island transducer induced by Ag NP binding to the APTS-modified silica overlayer. The small peak at 400 nm corresponds to the SP band of isolated Ag NPs between the Au islands, coinciding with the wavelength of the SP peak of the NPs in solution. The prominent effect of the Ag NPs on the extinction spectrum in the visible range is much greater than the dielectric response observed in the case of PPIX binding, indicating coupling between the Au and Ag surface plasmons. Possible aggregation of Ag NPs during drying may also contribute to the SP plasmon band at 650 nm.

Conclusions

Transmission localized surface plasmon resonance (T-LSPR) spectroscopy of evaporated Au island films presents a convenient platform for the construction of optical transducers for chemical and biological sensing. Formation of an ultrathin (∼1.5 nm) layer of silica on the metal islands stabilizes the optical response toward immersion in solvents, while maintaining the transducer sensitivity to analyte binding. The ultrathin, uniform silica layer was deposited at 90 °C for 2 h from a sodium silicate solution onto Au surface (islands or continuous film) precoated with a mercaptosilane monolayer. Formation of the silica coating was studied by ellipsometry and XPS analysis, while the integrity of the layer was evaluated by measuring the transport of electroactive ions in electrochemical experiments. CV data show facile transport of cations ($\mathrm{Ru(NH_3)_6}^{3+}$) and total blocking of anions ($\mathrm{Fe(CN)_6}^{3-}$), consistent with a defect-free, negatively charged nanoporous silica layer.

The optical properties of silica-coated Au island films are stable and virtually unaffected by interaction with various liquids, including PBS, an essential medium for biological applications. This allows preparation of T-LSPR recognition interfaces in which changes in the SP band are attributed exclusively to binding/release of analytes to/from the interface, fulfilling the basic requirement for sensing applications. The applicability and sensitivity of silica-coated Au island transducers were demonstrated by the detection of a porphyrin (PPIX) via electrostatic binding to a receptor layer on the silica surface, showing submonolayer sensitivity. Detection of Ag NP binding to a similar receptor layer on the silica revealed strong coupling of the Au and Ag surface plasmons.

We have previously shown that an unmodified T-LSPR recognition interface may be stable enough after solvent equilibration to enable biological sensing.[26] While this may be useful in certain cases, silica-stabilized T-LSPR transducers present a general route to the development of a wide variety of stable and sensitive optical sensors. The same methodology may be applicable to transducers prepared by immobilization of metal NPs on surfaces.

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