Ultrathin molecularly imprinted polymer sensors employing enhanced transmission surface plasmon resonance spectroscopy†

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An ultrathin novel nanosensor (31.5 ± 4.1 nm thick in the absence of analytes), employing a molecularly imprinted polymer as a recognition element for cholesterol and gold nanoparticle enhanced transmission surface plasmon resonance spectroscopy for detection, was constructed.

Molecular imprinting involves the fabrication of a recognition site for a desired small target molecule in a cross-linked polymeric matrix.[1–4] Molecularly imprinted polymers, MIPs, are prepared by using the target molecule as the template around which interacting monomers (and/or polymers) are polymerized, copolymerized (and/or cross-linked) to form a cavity-like recognition site. After polymerization (and/or cross-linking) the template is removed, and the binding sites become available for recognizing analytes which are complementary to the template in size, shape and position of the functional groups. Molecular imprinting is tantamount to creating a memory which is capable of selectively and reversibly rebinding the template (or the analogous analyte).[1–4] Efficient real time sensing requires the rapid diffusion of the analyte to and from the sensor. This can be accomplished by imprinting into nanoparticles[5] and pseudo two-dimensional ultrathin films.[6] The MIP sensor whose preparation and exploitation we report here was 31.5 ± 4.1 nm thick in the absence of analytes.

Charge density oscillations confined to coinage metal nanoparticles and nanoislands, referred to as localized surface plasmon (LSP) resonance, are exploited in transmission surface plasmon resonance, T-SPR, spectroscopy.[7–10] Excitation of LSPs by light at an incident wavelength where resonance occurs results in the appearance of intense surface plasmon (SP) absorption bands. The intensity and position of the SP absorption bands are characteristic of the type of material (typically, gold, silver, or platinum), the size, size distribution and shape of the nanostructures and highly sensitive to changes in the surrounding environments. This sensitivity was found to be further enhanced by attaching gold nanoparticles onto the terminal end of the attached molecules.[9–11] Recently, we reported that the T-SPR shift caused by the adsorption of double stranded DNA onto gold nanoislands is considerably enhanced by attaching 12 nm diameter gold nanoparticles onto the complimentary single stranded DNA used in the hybridization.[9] In a different work, using gold nanoparticle enhanced T-SPR we were able to monitor the pH change induced swelling and shrinking of an ultrathin polymer brush.[10]

The multilayered sensor was fabricated *via* five consecutive steps. Changes in the thickness were measured after each step of the sample preparation by AFM scratch tests (ESI Table 1†). We also prepared a reference sample of a similar multilayered structure on the surface of the Si-wafer (without gold nanoislands and without gold nanoparticles, to obviate light scattering) to monitor each deposition step by null-ellipsometry (see ESI Table 1†).

In step 1 gold nanoislands were deposited on the glass substrate (ESI Fig. 1†). The mean diameter of the gold nanoislands was assessed to be 14.5 ± 5.8 nm and the dominant center-to-center inter-island distance to be 35 nm. These values are in good agreement with those reported for nanoislands used in T-SPR spectroscopy.[9–11] Analysis of the height of the nanoisland layer obtained from of the AFM scratch test lead to a value of 10.1 ± 1.7 nm (see ESI Fig. 3 and Table 1†). In step 2 we spin-coated the polyglycidylmethacrylate (PGMA) anchoring layer (3.0 ± 0.6 nm from ellipsometry and 1.5 nm from AFM scratch test). The epoxy-functional group of the anchoring layer were used to graft carboxyl-terminated poly(2-vinylpyridine) (P2VP) in step 3. The P2VP layer thickness was estimated to be 3.8 ± 0.8 nm by the scratch test and 7.6 ± 0.8 nm by ellipsometry. The underestimation of the PGMA and P2VP layer thicknesses by AFM scratch tests can be rationalized by the "tip effect": the diameter of the AFM tip (20 nm in radius) is comparable to the inter-island distance, therefore, the several nanometres thick PGMA and P2VP layers were 'invisible' to the tip in the gaps between nanoislands (see ESI Fig. 1†). In step 4 the gold nanoparticles (12 nm in diameter) and cholesterol were adsorbed by the P2VP layer which then was cross-linked *via* a quaternization reaction with 1,4-diiodobutane to form the MIP layer. This step was performed in two versions: version 1—gold nanoparticles and cholesterol were adsorbed together, version 2—only cholesterol was adsorbed. The layer thickness was measured to be 22.9 ± 4.5 nm (scratch test) for version 1, and for version 2 to be 14.1 ± 1.9 nm (scratch test) and 15. 6 ± 2.1 nm (ellipsometry) (see ESI Table 1†). For version 1, gold nanoparticles were adsorbed again on the top of the cross-linked layer (step 5) (see ESI Fig. 2). The cavity-like recognition sites were formed in the P2VP MIP layer *via* washing off cholesterol molecules by rinsing the sample in ethanol then chloroform (for 3 min in each solvent). The cavities were reversibly filled with cholesterol at least 5 times. The addition of PGMA and P2VP layers, gold nanoparticles, cholesterol molecules and the removal of cholesterol was confirmed by taking the absorption spectrum of the dry sample after each corresponding

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step. The spectrum of the sample was identical after drying it with gaseous nitrogen for 15 s or leaving it to air dry for several days.

Thus, the samples consisted of the gold nanoislands, PGMA, P2VP MIP, cross-linked with or without gold nanoparticles. The cavities of the MIP were either empty or filled with cholesterol. Although the AFM scratch test and ellipsometry exhibited some discrepancies for the P2VP MIP layer, the changes in the thickness of the PVP MIP layer after introduction (at 1 mM concentration) and extraction of cholesterol were found to be similar for both techniques (8.0 nm by ellipsometry and 9.3 nm by scratch test, see ESI Table 1†) since the errors arising from the tip geometry are cancelled when subtracting thicknesses.

The sensor function is illustrated in Fig. 1. In the absence of cholesterol the localized surface plasmon band of the interacting gold nanoislands and nanoparticles has an absorption maximum at 654 nm. Introduction of cholesterol into the cavity-like recognition sites of the MIP results in a marked change of the overall reflectance of the stratified layers which constitute the sensor. This manifests itself in a 56 nm shift of the absorption maximum to 598 nm.

We have also determined the shift of the absorption maximum of the interacting gold nanoislands and nanoparticles in the absence and presence of structurally similar stigmasterol, digitoxigenin and progesterone. The markedly smaller shifts (14 nm for stigmasterol, 26 nm for digitoxigenin and 30 nm for progesterone) than that observed for cholesterol (56 nm) show a reasonable degree of selectivity of the MIP sensor considering that no attempts have been made for optimization. This selectivity was lost when we carried out identical experiments employing a sensor prepared by a lesser degree of cross-linking of the MIP (using lower concentrations of the cross-linker), thus substantiating molecular recognition in the MIP originating, most likely, in hydrogen bonding interactions between the nitrogen groups of pyridine in the polymer and the OH groups in the cholesterol (and in its analogs). Cross-linking, of course, stabilizes the spatial location of pyridine moieties in the polymer, hence it provides the molecular recognition needed for selectivity of the MIP. It has to be mentioned, however, that non-specific swelling of the polymer, induced by the insertion of the analytes into the film, or aggregation of cholesterol with traces of template not removed in the washing procedure may contribute to the shift of the absorption maximum of the interacting gold nanoislands and nanoparticles.

The repeatability and reproducibility of the experiments (five successive introductions and washings of cholesterol gave absorbance shifts of 56 $\pm$ 2 nm) suggest that this technique is suitable for the construction of multiple use sensors. The speed of sensing (the optical changes occurred within seconds when exposing the samples to the analyte) permits the use of *in situ* real time sensing by our sensor.

Polarization modulated Fourier transform infrared reflection absorption spectroscopy (PM-FTIRRAS) was used to confirm the presence of cholesterol in the MIP sensor. Samples for PM-FTIRRAS were prepared on the surface of 100 nm thick continuous gold films (instead of the gold nanoislands). Each step of the procedure (deposition of PGMA, deposition of the P2VP, cross-linking to form the MIP in the presence of cholesterol, washing out the cholesterol, and adding the cholesterol as an analyte; see ESI Fig. 4 and experimental details†) was followed by a PM-FTIRRAS. Cholesterol was introduced into the cavities of the MIP sensor by exposing the sample to a 1 mM and a 10 mM solution of cholesterol in chloroform for 1 min. The differential spectra, due only to cholesterol, provide for the effective functioning of our sensor. The peaks at 2940, 2860, 1465 and 1381 cm^{-1} are the characteristic vibrations of methyl groups, as indicated in Fig. 4 in the ESI.†

The signal enhancing property of gold nanoparticles is demonstrated by comparing the T-SPR spectra of MIPs containing interacting gold nanoislands and nanoparticles (A in Fig. 2) with those containing gold nanoislands only (B in Fig. 2) and gold

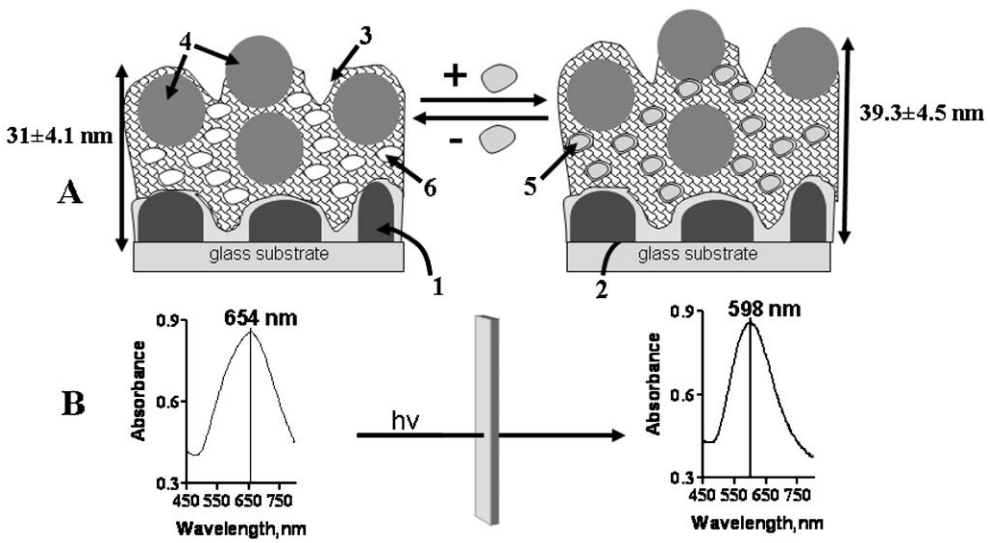

Fig. 1 (A) Schematic illustration of the ultrathin molecularly imprinted polymer (MIP) sensor employing gold nanoparticle enhanced surface plasmon resonance spectroscopy: the stratified layers of gold nanoislands (1), PGMA binder (2), MIP (3), and gold nanoparticles (4) are illustrated in the absence and in the presence of the analyte (5) (cholesterol) which occupies the cavity-like recognition sites (6). (B) Transmission surface plasmon resonance T-SPR spectrum of the interacting gold nanoislands and gold nanoparticles in the absence (left hand side) and presence (right hand side) of cholesterol. The schematics of T-SPR spectroscopy is shown in the middle.

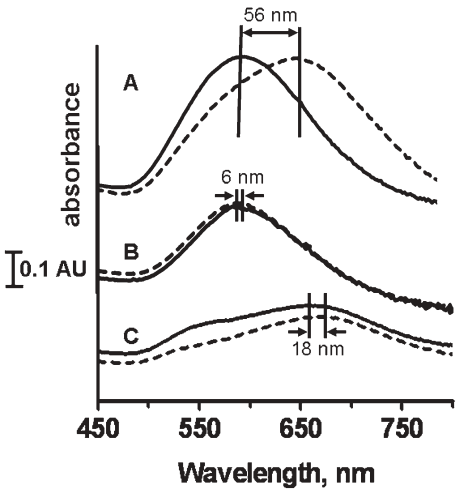

Fig. 2 (A) Transmission surface plasmon resonance T-SPR spectra of MIP with interacting gold nanoislands and gold nanoparticles; (B) gold nanoislands only; (C) and gold nanoparticles only in the absence (broken lines) and in the presence of cholesterol (solid lines).

nanoparticles only (C in Fig. 2) in the absence and presence of cholesterol. Both the absorbances (0.45 for A, 0.32 for B and 0.16 for C) and the shift of the absorption maximum (56 nm for A, 6 nm for B and 18 nm for C) are significantly smaller for just the gold nanoislands (B in Fig. 2) or just the gold nanoparticles (C in Fig. 2) than those for the situation when both are present in close proximity (A in Fig. 2) and in synergy with the localized surface plasmon resonance by their interactions.

In this communication we have reported our initial results on the fruitful combination of MIPs and gold nanoparticle enhanced T-SPR spectroscopy. Specifically, we described the construction of a 31.5 $\pm$ 4.1 nm thick nanosensor employing a MIP as a recognition element for cholesterol and the use of T-SPR spectroscopy for detection and gold nanoparticles for signal enhancement. The significance of this work is manifold. First, it employs an ultrathin MIP which permits the detection of the analyte in real time. Second, gold nanoparticle enhanced T-SPR spectroscopy is highly sensitive, versatile and economical. Absorbances of the order of 0.3–1.0 are routinely obtained by simply placing single layers of interacting (but not touching) 10 nm thick gold nanoislands and 12 nm diameter gold nanoparticles on a glass slide into a standard spectrophotometric cell. We have shown here that the introduction of cholesterol into the MIP results in a 56 nm shift of the absorption maximum of the interacting gold nanoislands and nanoparticles which sandwich the MIP.

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