Supporting information for

Attomolar protein detection using in-hole Surface Plasmon Resonance

Jacqueline Ferreira,[1,2] Marcos J.L. Santos,[1,2] Mohammad M. Rahman,[1] Alexandre G. Brolo,[*,1] Reuven Gordon,[3] David Sinton,[4] Emerson M. Girotto[2]

[1] *University of Victoria, Department of Chemistry, P.O. Box 3065, V8W 3V6, Victoria, BC, Canada.*

[2] *Universidade Estadual de Maringa, Av: Colombo 5790, 87020900 Maringa, Parana, Brazil.*

[3] *Department of Electrical and Computer Engineering, University of Victoria, BC, V8W 3Z8, Victoria, BC, Canada.*

[4] *Department of Mechanical Engineering, University of Victoria, BC, V8W 3P6, Victoria, BC, Canada.*

1.1 Preparation of the SiO_x coating layer

1" x 1" gold-coated glass slides, obtained from EMF, were used. A 5 nm Cr layer deposited on the glass enables the adhesion of the 100 nm Au film to the substrate. The procedure for the deposition of thin SiO_x nanolayer on these 100 nm-thick gold films on glass substrates was as following: The gold slide was mounted in a 15 mL Teflon cell, leaving a surface area of *ca.* 1 cm^2 of the gold side exposed. An adhesion layer of 3-Mercaptopropyl(trimethoxysilane) (MPTMS) was used to attach the SiO_x layer to the gold surface. The MPTMS solution was added to the exposed gold surface in the Teflon cell and it was allowed to stay for 24 hours. Even considering that the initial adsorption of MPTMS is fast, the organization phase was allowed to continue for 24 hours in order to obtain a high degree or order in the monolayer. After the MPTMS self assembled monolayer was formed, a 0.54 wt % sodium silicate solution was added to the cell and it immersed the gold surface for 120 hours, resulting in a nanolayer of SiO_x. The thickness of the SiO_x film (50 nm) was then determined by AFM. This procedure for SiO_x deposition was based of the previous description for the encapsulation of gold nanoparticles by a SiO_x nanolayer reported by Mulvaney P. *et al*. [1-3].

1.2 Characterization of the blocking properties of the SiO_x layer

The blocking properties of the SiO_x layer was characterized by cyclic voltammetry (CV) experiments using 1 mM $K_3[Fe(CN)_6]$ in 0.1 M KCl. The gold slides were used as working electrodes. A Pt wire and an Ag½AgCl were used as counter and reference electrodes, respectively (all potentials reported here are against the Ag½AgCl½0.1 M Cl^- reference electrode). Figure S1 shows the CVs from a bare gold slide (Fig. S1(a)) and from a gold slide coated with a 50 nm layer of SiO_x (Fig. S1(b)). At bare gold, the redox process of the $[Fe(CN)_6]^{4-}$ /$[Fe(CN)_6]^{3-}$ pair is evident by an oxidation peak at 0.34 V and a reduction peak at 0.18 V. After the formation of a 50 nm thick SiO_x film, the current decreases dramatically due to the limited diffusion of $[Fe(CN)_6]^{4-}$/$[Fe(CN)_6]^{3-}$ species toward the gold surface. The curve in Fig. S1(b) does not present waves of sigmoidal form as earlier reported in the literature [4,5]. Fig. S1(b) indicates that the redox process has been completely blocked by the SiO_x layer. The residual current observed in this CV has been attributed to stray capacitance.

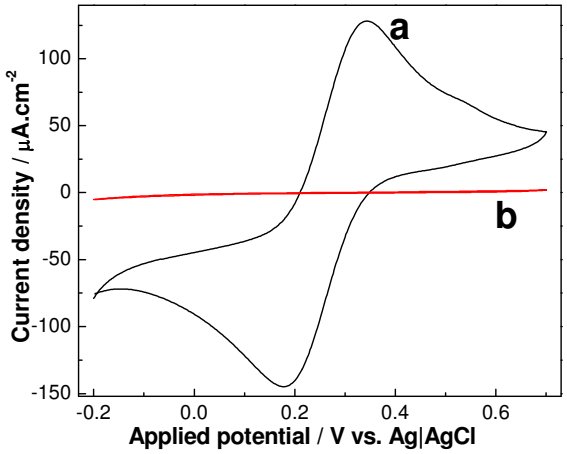

Figure S1. CVs of 1mM of $K_3[Fe(CN)_6]$ in 0.1 MKCl at (a) bare gold slide, and (b) gold slide coated with a 50 nm SiO_x film obtained after 120 hours of deposition.

1.3 Fabrication of the arrays of nanoholes

The arrays of nanoholes were fabricated by focused ion beam (FIB). The nanoholes were milled all the way through the 50-nm thick SiO_x film, the 100-nm gold film and the 5 nm Cr adhesion layer. The nanoholes were *ca.*170 nm in diameter, and the array periodicity was 500 nm. The SEM image of a typical array is shown in Figure S2(a). An expanded view is presented in Figure S2(b). 16 arrays were investigated. Each nanohole array contained 30 x 30 nanoholes and occupied an area of *ca.* 15 µm x 15 µm. The footprint of the entire set of arrays was around 0.2 mm x 0.2 mm.

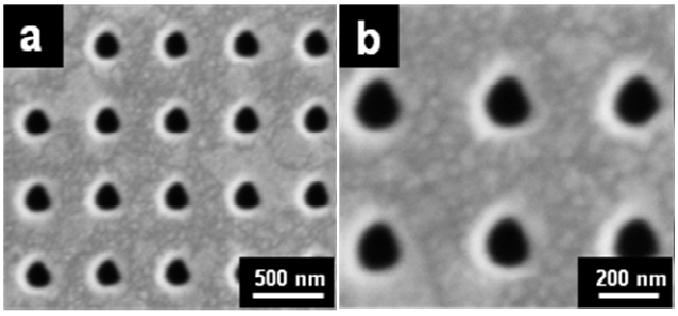

Figure S2. SEM images of the array of sub-wavelength holes (a and b)

1.4 Surface modification (streptavidin immobilization):

The cysteamine-biotin-streptavidin system is well established as a model to evaluate the performance of SPR [6]. Cysteamine was used as an adhesive layer because it presents a thiol group that binds irreversibly to the gold surface [7,9].The immobilization of streptavidin is obtained in a three step process. First, a 6.2 mM cysteamine solution was prepared. The arrays of nanoholes were rinsed with acetone and methanol, placed in an ultrasonic bath in methanol for 3 min, plasma cleaned for 15 min, and immersed in the cysteamine solution for 72 h to assemble a monolayer on the gold surface. The gold chip was removed from the cysteamine solution after the monolayer formation and cleaned with ethanol and with distilled water, and then assembled in a flow cell. In the second step, a solution of biotin linker ((+)-biotin N-hydroxy-succinimide ester) was prepared by dissolving 12 mg of biotin into 2 mL of dimethyl sulfoxide (DMSO). This biotin solution was introduced to the cysteamine monolayer via flow cell for 45 min at a rate of 0.02 mL/min and then the cell was flushed with PBS buffer solution. The third and final step in the protein binding experiment involved the streptavidin protein solution (1 mg of streptavidin dissolved in 4 mL of PBS buffer). The nanohole arrays were exposed to the streptavidin solution for 15 min at a flow rate of 0.02 mL/min.

.

References for supporting information:

(1) Ung, T.; Liz-Marzan, L.M.; Mulvaney, P. *Langmuir* **1998,** *14*, 3790.

(2) Ung, T.; Liz-Marzan, L.M.; Mulvaney, P. *J. Phys. Chem. B* **2001**, *105*, 3441.

(3) Mulvaney, S. P.; Musick, M. D.; Keating, C. D.; Natan, M.J. *Langmuir* **2003**, *19*, 4784.

(4) Shen, Y.; Wu, T.; Zhang. Y.; Li, J. *Talanta* **2005,** *65*, 481.

(5) Szunerits, S.; Boukherroub, R. *Electrochem. Commun.* **2006,** *8*, 439.

(6) Neuert, G.; Kufer, S.; Benoit, M.; Gaub, H. E. *Rev. Sci. Instrum.* **2005**, *76*, 054303.

(7) Wirde, M.; Gelius, U. *Langmuir* **1999,** *15,* 6370.

(8) Katz, E.; Lotzbeyer, T.; Schlereth, D. D.; Schuhmann, W.; Schmidt, H.-L. *J. Electroanal. Chem.,* **1994**, *373*, 189.

(9) Mirkhalaf, F.; Schiffrin, D. J. *J. Chem. Soc., Faraday Trans.,* **1998**, *94*, 1321.