Supporting Information

Label free detection of specific protein binding using a microwave sensor

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Experimental

Materials

3,3'-Dithiodipropionic acid di(N-hydroxysuccinimide ester) (DSP), dimethyl sulfoxide (DMSO), protein A–biotin from *Staphylococcus aureus*, 3,3’,5,5’-tetramethylbenzidine (TMB) peroxidase substrate, Tween-20, cytochrome c and glucose oxidase were purchased from Sigma-Aldrich. Streptavidin-horseradish peroxidase and ethanolamine hydrochloride were purchased from GE Healthcare Lifesciences. Hydroxyapatite powders were obtained from Cambioceramics. All solutions were prepared using ultrapure water (resistivity of 18.2 MΩ cm) prepared using an Elgastat maxima system (Elga, UK).

Substrate Preparation

One side of silicon wafer slides (5 mm x 7 mm) were sputtered with a layer of titanium (~2 nm) followed by a layer of gold (~ 50 nm) using an ORION-5-UHV sputtering system. The gold slides were cleaned in (70 % v/v) nitric acid for 15 min at room temperature. The substrates were thoroughly rinsed with ultrapure water and air dried.

Gold surface modification

The clean gold substrates were immersed in 2 mM DSP in DMSO for 2 h at room temperature in order to create a thiol layer. The slides were then rinsed with DMSO, followed by rinsing with a solution of phosphate buffered saline (PBS, 10 mM sodium phosphate, 137 mM NaCl at pH 7.2). The protein A layer was covalently attached to the thiol linked gold substrates by incubating the slides overnight at 4 °C in a protein A-biotin solution (0.5 mg/ml) in PBS at pH 7.4. The surfaces were then blocked by incubation in a solution of ethanolamine hydrochloride (1M), pH 8.6, for 1 h. After thorough washing with PBST (PBS with 1% Tween 20, pH 7.2) the substrates were incubated in a PBST solution containing 1.2 µg/ml streptavidin-HRP for 2h. Thiol modified gold substrates were blocked with a solution of ethanolamine hydrochloride (1M), pH 8.6, for 1 h and then incubated in 1.2 µg/ml streptavidin-HRP in PBST solution for 2 h as a control. 3,3’,5,5’ Tetramethylbenzidine (TMB) peroxidase substrate was added as a substrate for the detection of HRP.

Hydroxyapatite film preparation

Hydroxyapatite (HA) powders were mixed with a binder polyvinyl butyral (PVB) together with diethylene glycolbutyl-ether as solvent. The HA paste was then smeared over glass slides using a glass microscope slide as a squeegee. The obtained films were dried at 200°C for 10-15 min and then calcined (ramp rate of 5°C/min and for 1 hour at 700°C) to remove the solvent and the binder.

HA surface modification

HA films were immersed in a 20 µM solution of cytochrome c in 10 mM KH2PO4 buffer, pH 7.0 and in a 20 µM solution of glucose oxidase in 10 mM C2H3NaO2 buffer, pH 5.0.

SEM characterization
SEM images of the HA films were obtained using a Hitachi SU-70 (accelerating volt of 10 keV) equipped with an energy dispersive X-ray spectrometer. Thin films of gold were deposited on HA films to reduce charging effects.

**Microwave measurements**

A pattern with interdigitated electrodes (IDE) printed on Rogers® substrate was attached to a Rohde and Schwarz ZVA24 vector network analyzer (VNA) via a coaxial cable as described previously. The reflected signal ($S_{11}$) was recorded for all samples (60,000 points for each measurement) over the frequency range 0.01-15 GHz. All measurements were made in air.

![Graph showing reflected signal ($S_{11}$) response](image)

Fig. S1. Reflected signal ($S_{11}$) response obtained for a thiol modified gold surface which was subsequently modified with protein A-biotin and with streptavidin-HRP.
Fig. S2. Reflected signal $(S_{11})$ response obtained for a thiol modified gold surface which was subsequently modified with protein A-biotin and with streptavidin-HRP.
Fig. S3. Reflected signal ($S_{11}$) signal response obtained for a HA modified gold surface with bound glucose oxidase and cytochrome c.