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Label free detection of specific protein binding using a microwave sensor†

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The specific binding of streptavidin to biotinylated protein A was demonstrated using a microwave detection system. In control experiments, the degree of non-specific binding was negligible. The method of detection was used to monitor the adsorption of two other proteins, cytochrome c and glucose oxidase, on to the IDE microwave sensor surface. The response of the sensor was also examined on different substrate materials, with detection of protein binding observed obtained on both smooth, conductive (gold) and on rough, insulating (hydroxyapatite) surfaces.

Interest in developing methods to detect protein binding has grown in the last decade. Current sensing techniques fall into two classifications; label-based or label-free. The former uses labels to monitor binding[1] using detection methods such as fluorescence, absorbance, chemiluminescence or radiolabelling. Techniques such as enzyme-linked immunoassay (ELISA) enable both the detection of the presence of protein in a sample as well as the amount of protein bound to a given antibody. ELISA is a highly sensitive method; yet the use of labels can require complex sample preparation and long analysis times.[2, 3] In addition, the use of labels can require chemically modification of the sample that may subsequently interfere with the binding site of the analyte of interest.[1]

Surface plasmon resonance (SPR) is a label free optical method that uses the reflectivity of a thin metal layer (typically gold) to measure changes in the refractive index. Changes in the SPR signal occur when species bind to the surface or to an immobilized target. These changes provide information related to the association and dissociation phases of the reaction.[2, 4] In comparison to other techniques, the advantages of SPR include specificity, sensitivity and rapid detection times[5]. The specificity is provided by a biological recognition system. The main disadvantage of SPR is the poor sensitivity when monitoring ligands with low molecular weight.[6, 7]

The measurement of mass changes using a quartz crystal micro balance (QCM) is an alternative label free technique commonly used for the detection of bound analytes to a surface.[8] The utilization of QCM with dissipation monitoring can enable label-free and quantitative analysis of binding events in real time.[9] However its use in quantitative analysis in the liquid phase can be challenging since the measurement step can also incorporate mass changes associated with solvent molecules coupled to the adsorbent.[10]

Electrochemical impedance spectroscopy (EIS) is another label free form that is used to detect the binding of biomolecules. Impedance sensors measure electrical impedance changes by applying an AC potential and the selectivity in EIS is accomplished by using a functionalized surface to impart specificity to the method.[11] The main challenge in EIS methods is obtaining selectivity in complex samples.[12]

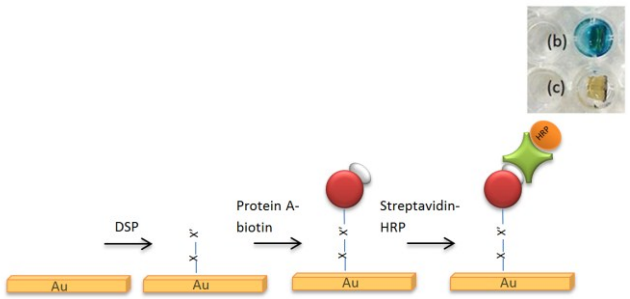
Microwave sensing measures the response of a material in the GHz frequency range. When a material is in contact with microwave radiation, it will interact with the radiation in a manner that results in a frequency change, *i.e.* attenuation or reflection of the signal. The frequency changes observed depend on the structure and molecular masses of the molecule under examination.[13]

Microwave detection can be used to monitor changes in the dielectric response of an analyte due to reflection (S_{11}) or transmission (S_{21}) of the microwave radiation. An advantage of using microwave techniques for material characterization is that microwave radiation can propagate through the material. Moreover, the amplitude of the electromagnetic wave reflected by or transmitted through a material is largely dependent on the dielectric properties (permittivity or conductivity) of the material under test. The reflected signal depends on the material under test and changes in this signal arising from

changes in the permittivity can be linked to the composition of the material. The permittivity is a measure of how an electric field is affected by a dielectric medium and accounts for both the stored energy (ε') as well as the loss of energy (ε'') within the material. Signature spectra can be obtained and changes can be monitored within a range of frequencies, indicating that chemical changes that lead to a change in the complex permittivity[14] can be detected using this technique. This is often accomplished by using specifically designed microwave sensing structures (an antenna or a cavity) that enable the measurement of the dielectric response of an analyte within the GHz frequency range.[15] The obtained spectra depend on the design of the sensing structure.

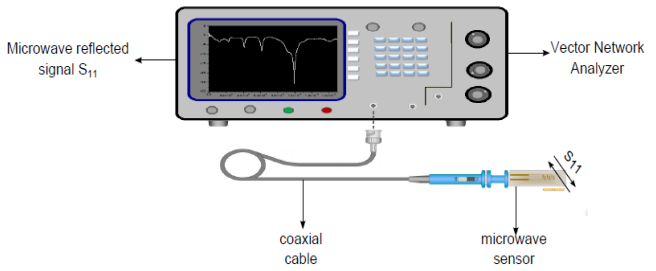
Microwave detection has been used in applications such as the non-destructive evaluation of activated carbon[16] and the detection of glucose.[17] These studies focused on sensitivity and utilized unselective detection methods. The development of label-free detection methods is of significant interest in the field of sensors. In this work we describe a label free microwave detection technique that can be used to qualitatively detect specific binding of streptavidin to biotinylated protein A (PA-biotin).

Gold substrates were modified as shown in Scheme 1. An IDE type sensor operating at microwave frequencies was placed on top of the modified substrates (Scheme 2). The sensitivity of IDE sensors can change close to the sensor surface; this feature has been previously demonstrated using finite element modeling.[18] Avidin-biotin binding has been extensively examined as a model system for protein-ligand interactions.[19] DSP forms a self-assembled monolayer on the gold surface and can subsequently react with primary amines,[20] such as those present on protein A, anchoring the protein to the surface. Streptavidin (SA) conjugated with horseradish peroxidase (HRP) was utilized to visually confirm the attachment of SA-HRP to the biotinylated protein A layer. On exposure of the surface to a solution of tetramethylbenzidine (TMB) and hydrogen peroxide, a blue reaction product formed, (scheme 1(a)) indicating that binding of SA-HRP to the PA layer on the surface had occurred.



Scheme 1. (a) Schematic representation of the immobilization of protein A-biotin and streptavidin-HRP on a gold surface modified with DSP for microwave sensing. Image of (b) the blue reaction product obtained on binding of streptavidin-HRP to a protein A-biotin layer immobilized on a coated gold surface and (c) the response of a control experiment in the absence of protein A-biotin on the gold surface.

Spectra of the IDE microwave sensor in contact with the modified gold substrate were recorded in the 0.01-15 GHz frequency range. Fig. 1 illustrates the S11 response (0.01-15 GHz) obtained for three separate samples modified with PA-biotin. No significant shift in resonance frequency was observed indicating that a reproducible signal was obtained from each surface. The surface area occupied by a protein A molecule[20] is estimated as ca. 78.5 nm^2 corresponding to a surface coverage of ca. 2 pmol cm^{-2} (assuming a full monolayer coverage).



Scheme 2 Schematic representation of sensor

Changes in the response of a microwave sensor arise from changes in the permittivity of the material and the rotational states of the molecules under examination. A change in peak intensity is indicative of the presence of the protein. Shifts in the resonant frequency arise from changes in molecular composition e.g. binding to another molecule. It should be noted that microwave sensors are highly sensitive to dielectric changes[21, 22] and measurements in liquid have been reported for the detections of glucose. [21, 23] The challenge in using this approach is to enable specific and selective detection of the binding of an analyte to the surface. Such selective detection can be achieved by attaching a specific binding element to the surface. Here biotinylated PA is used to provide the sensitivity required to specifically detect SA. Fig. S1 illustrates the S_{11} signal response obtained for PA-biotin modified with streptavidin-HRP.

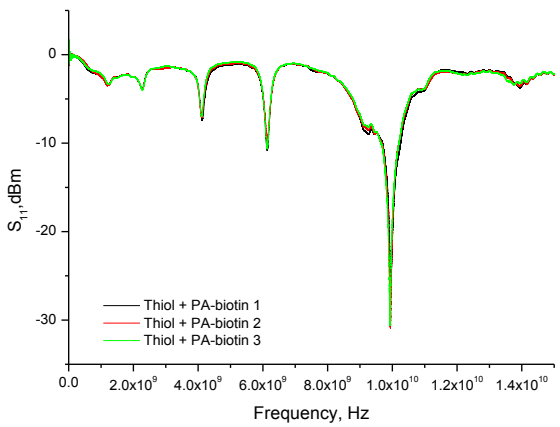


Fig. 1. Reflected signal (S_{11}) obtained with three individual PA-biotin modified gold samples.

A significant change in the signal frequency was observed when SA-HRP was added to the surface and an additional peak was observed at a frequency of 9.23 GHz (Fig. 2). The frequency range of 8-12 GHz was selected as it showed a change in frequency that depended on the nature of the sample in contact with the biosensor. The response obtained is presumed to arise from permittivity changes occurring due to the specific binding of SA-HRP to the biotinylated surface.

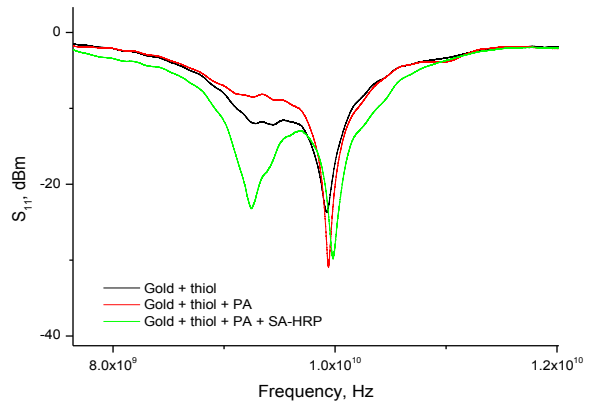


Fig. 2. Reflected signal (S_{11}) of the IDE microwave sensor in contact with a protein A-biotin surface modified with streptavidin-HRP over the frequency range 8 – 12 GHz

To verify that the changes in frequency arise from the streptavidin–biotin binding, control experiments were conducted. The degree of non-specific binding was examined by blocking the thiol modified gold substrates with ethanolamine hydrochloride, followed by incubation with a solution of SA-HRP (1.2 µg/ml). Control samples were immersed in a solution of TMB and hydrogen peroxide, and did not yield any color change (Scheme 1 (c)). Fig. 3 illustrates the S_{11} signal response of an IDE microwave sensor in the 0.01-15 GHz region in contact with DSP modified substrate with SA-HRP. The spectra obtained are very similar. Changes in the concentration of a material will alter the permittivity; thus the differences observed are likely due to nonspecific protein binding of SA-HRP to the thiol surface. Similar responses for two separately prepared samples were obtained (Fig. S2).

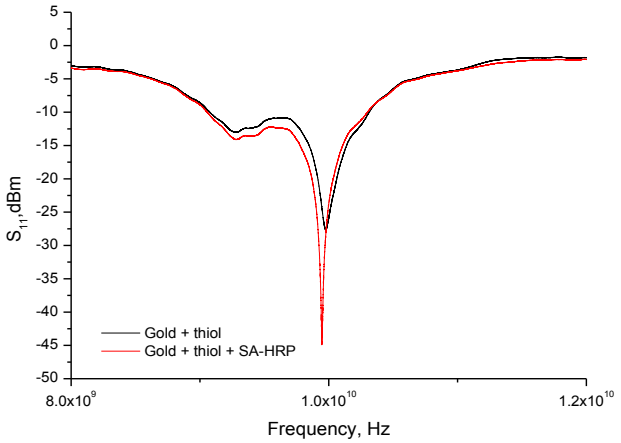


Fig. 3. Reflected signal (S_{11}) obtained in the 0-15 GHz range for a thiol sample modified with SA-HRP.

Although SPR has emerged as the most commonly used technique to examine for protein-protein interactions,[24] a limitation in its utilization is the requirement of a smooth, conductive metal surface to generate the surface plasmon wave. The microwave detection method described here can be advantageous because binding interactions can be selectively monitored on insulating substrates with rough morphologies. The binding of the proteins (cytochrome c and glucose oxidase) onto the inorganic mineral hydroxyapatite (HA) was detected using the microwave method. HA is a ceramic, and the main inorganic component found in vertebrate bone, dentin and enamel. HA is biocompatible, non-toxic, chemical stable and is bioactive. Microwave measurements were recorded in the 0.01-15 GHz range and the obtained spectra was very similar for the two proteins (Fig. S3). The SEM micrograph in Fig. 4(a) shows that HA has a spherical and rough porous morphology. Fig. 4(b) illustrates the S11 signal measured in the 8-12 GHz range where there is a distinctive shift in the resonant frequencies. The spectra obtained for both cytochrome c and glucose oxidase are similar. It is worth noting that changes in the resonant frequency for the all proteins analyzed (cytochrome c, glucose oxidase and SA) occurred at a similar frequency range (9.0 – 10 GHz). The spectra recorded for HA were different to the spectra obtained for proteins immobilized on HA, with changes observed in both the frequency and the number of resonant peaks. This confirms the ability of the technique to monitor protein binding on various surface materials. As described above, a biological recognition element is required to provide selectivity to the method.

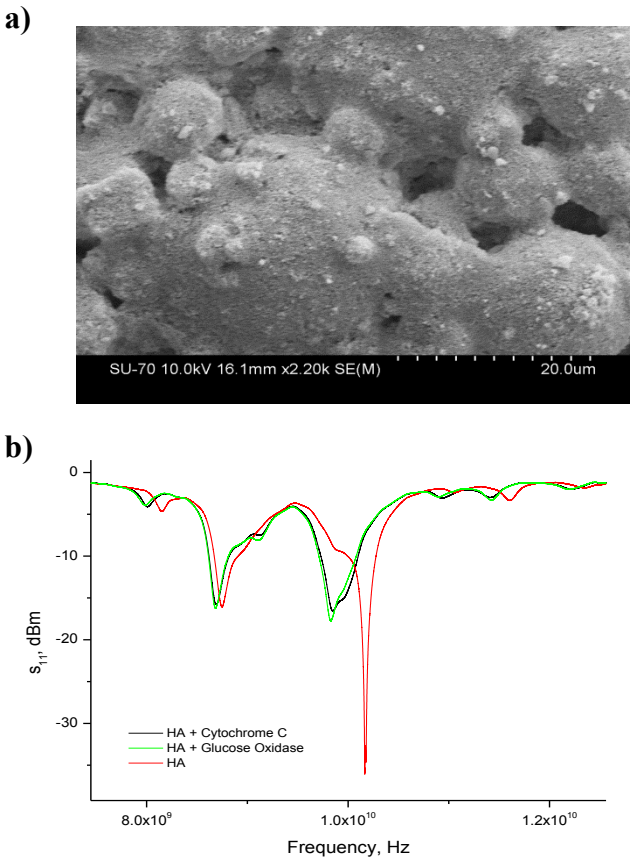


Fig. 4. (a) SEM of HA film on a coated gold surface and (b) reflected signal (S_{11}) obtained with a hydroxyapatite modified surface and on exposure to cytochrome c and glucose oxidase.

Conclusions

The ability to detect specific protein binding using a microwave detection method has been demonstrated using streptavidin and biotinylated protein A. Minimal non-specific binding was observed. The method requires the presence of a specific binding element to enable selective recognition of binding. Measurements can be performed on rough or smooth surfaces and the support can be an insulator or a conductor. The system described here illustrates the potential of microwave analysis for the qualitative detection of protein-ligand interaction; quantitative analysis has yet to be further examined. The development of a flow system is currently in progress to evaluate the response of the sensor in real time.

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Notes and references

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