

# Ultrasensitive Protein Detection Using an Au/ZnO SPR-Based Biosensor

C.-C. Chang<sup>\*</sup>, S.-H. Lin<sup>\*\*</sup>, N.-F. Chiu<sup>\*\*\*</sup>, C.-W. Lin<sup>\*\*\*\*</sup>

<sup>\*</sup>National Taiwan University, Taipei, Taiwan, ROC, ccchang.ibme@gmail.com

<sup>\*\*</sup>National Yang-Ming University, Taipei, Taiwan, ROC, dslin1511@yahoo.com.tw

<sup>\*\*\*</sup>National Taiwan University, Taipei, Taiwan, ROC, nfchiu@ntu.edu.tw

<sup>\*\*\*\*</sup>National Taiwan University, Taipei, Taiwan, ROC, cwlinx@ntu.edu.tw

## ABSTRACT

In this study, we demonstrated that the Au/ZnO nanocomposite films could be effectively used to enhance the performance of SPR for the detection of tumor marker. CA15.3 tumor marker for breast cancer was chosen as a model analyte. We analyzed experimentally intensity response to the samples that had different concentrations (0.0125 U/ml to 160 U/ml) in pleural fluid to evaluate the detection capability of the Au/ZnO SPR-based biosensor. As a result, it was found that the linear range extended from 0.025 to 40 U/ml with correlation coefficient of  $R = 0.9531$  and the limit of detection at S/N of 3:1 reached 0.025U/ml. Compared with the shift degree of SPR intensity by the specific binding event between antibody and antigen, the change of intensity on the Au/ZnO layers increased at least 2 fold more than that on the Au/Cr layers. In addition, we determined that the Au/ZnO layers had a detection limit 4 times lower than the Au/Cr layers that were widespread use of the sensing interfaces on SPR. In conclusion, the use of Au/ZnO films greatly enhanced the SPR signal after carrying out bimolecular interactions and presented high sensitivity and reproducibility.

**Keywords:** ultrasensitive, Au/ZnO, tumor marker, Carbohydrate antigen 15.3

## 1 INTRODUCTION

In wide variety of biosensors, it is crucial to measure the concentrations of specific proteins in biological fluids. Numerous studies in recent years have focused on the improvement of the sensitivity of biomolecular detection such as tumor marker[1], virus[2], and bacteria[3]. Many detection assays rely on the biomolecular recognition with fluorescence, radioisotope, and other types of label methods. However, they have several drawbacks such as low selectivity and conformational changes [4, 5]. Thus, an optical detection on the surface plasmon resonance (SPR) response that has increased in popularity is provided. SPR is advantageous compared to label techniques because it does not require any special labels and can detect directly.

In the most frequently used fabrication for SPR chips, gold films are deposited on top of thin layer of chromium.

The characteristics of chromium thin films play an important role because they can greatly improve the adherence between the gold layer and the glass substrate. Nevertheless, the unique optical properties of nanomaterials can be act as the innovation biosensor substrates for enhancement of the detection sensitivity. In recent years, ZnO has received increasing attention because it exhibits many interesting properties including wide band gap, optical transparency in the visible region, high refractive indices, large piezoelectric constants, and nonlinear optical coefficients[6]. It has been used as transparent electrodes and thin film transistors for acoustic wave devices, gas sensors, and piezoelectric transducers. In addition, it can be considered as a potential candidate for biocompatible material in many biomedical applications[7]. Recently, many studies have carried out in exploring Au/ZnO nanocomposites because of their desirable optical properties. Nevertheless, the applications of Au/ZnO nanoscale thin films in biosensors have not yet been comprehensively realized.

Herein, we report the use of the Au/ZnO thin films in the enhanced SPR detection. Carbohydrate antigen 15.3 (CA15.3) is a promising candidate biomarker for metastatic breast cancer, which is chosen as a protein model. We show that Au/ZnO nanocomposite materials exhibit a great optical property in promoting detection at ultra-trace concentrations. Furthermore, enhanced detection limits of Au/ZnO thin films in the identification of a breast tumor marker were determined and compared with those for Au/Cr thin films.

## 2 EXPERIMENTS

### 2.1 Analysis of bare gold surface

The morphology of the surface was measured by means of atomic force microscopy (AFM). The AFM images were carried out in contact mode in  $5 \times 5 \mu\text{m}^2$  on samples.

### 2.2 Fabrication of SPR Chip

We fabricated two different sensing substrates including Au/Cr and Au/ZnO. All of these thin films grown on SF10 glass substrate are to be seen in Fig. 1. All Cr and Au films were deposited by electron beam evaporator at a vacuum level of about  $3 \times 10^{-6}$  Torr. The ZnO film was grown on SF10 glass substrate by using a radio-frequency (RF) 13.56 MHz sputtering system. A working pressure of 3 mTorr was employed during the deposition and the mixture of Ar (40 %) and O<sub>2</sub> (30 %) was used as the working gas.

### 2.3 Preparation of the sensor surface

The schematic diagram of the surface fictionalization was depicted in Fig. 2. The assay developed was designed as a bio-affinity immobilization assay. The gold-coated slide was immersed into 8-Mercaptooctanoic acid (8-MOA) solution at room temperature for 20 minutes. Then the chemical immobilized surface was activated using 400 mM EDC/100 mM NHS for covalent bond formation in 10 minutes at room temperature. The gold film chip soaked in 50 µg/ml protein G solution which fixed on the gold surface for 10 minutes. The antibody at concentration of 5 µg/ml was immobilized onto the surface for 20 minutes and the surface was blocked with 1% BSA for 10 min.

### 2.4 SPR measurements

At the starting step, the gold surface was exposed to PBS buffer at a flow rate of 40 µL/min in order to get the stable baseline. The Au/Cr and Au/ZnO chips were prepared to measure the intensities of reflected light by using SPR at the respective angle of 50.5 and of 51 degrees under 790nm wavelength. CA15.3 at different concentration from 0.0125 U/ml to 160 U/ml in PBS were injected into the immobilized surface at the above flow rate. PBS was finally added to the immobilized surface followed by buffer rinse.

## 3 RESULTS AND DISCUSSION

### 3.1 Characterization of sensor surfaces

The performances of conventional SPR biosensors are well known to depend not only on the optical properties of the optical components but also the morphology details of the different interfaces, mainly the free surface. A flat bare gold surface plays an important role in the stability of SPR measurements. To investigate the surface morphology, AFM was used for the topographic characterization of

Au/Cr and Au/ZnO nanocomposite films. Fig. 3 showed an AFM image of the Au/Cr (Fig.3a) and of the Au/ZnO bare gold surface (Fig.3b). It could be seen that the topographies were both no large features and the variation of roughness were within the 2 nm scale. Therefore, the result was indicated that the possibility of unstable SPR signal due to the roughness of bare gold was eliminated.

### 3.2 Detection of CA15.3 by the Au/ZnO thin films SPR

To achieve the high sensitivity and specificity of immunoassays, it is critical for the immobilization process of antibodies to have corrected orientation to access their active sites for intended functions on the sensor surface. Antibodies in a highly oriented manner have been shown to maintain higher binding capacity than those of random manner[8]. Protein G, which is surface protein from streptococcal strain, can lead to immune escape because it is generally expected to bind to the Fc part of antibody specifically[9]. This provides specific affinity binding sites for interaction with antibodies. The possibility of any functional damage to antibodies due to random coupling is also eliminated. Hence, to obtain higher sensitivity, the bio-affinity immobilization was adopted for the assay using the protein G.

For detection of CA15.3, sample concentrations of 0.025 U/ml and above resulted in significant intensity change and saturation of response was obtained at 40U/ml. The SPR intensity of control had an average value of  $0.02 \pm 0.13$  au. On the definition of the limit of detection (LOD), it was determined as three times the standard deviation of the control. Hence, the LOD was 0.025 U/ml because it contained an average value of  $0.60 \pm 0.23$  au. This assay was more sensitive than previous reports for tumor marker detection. The linear dynamic range was from 0.025 U/ml to 40 U/ml, in which the data points were linearly fitted with least squares approximation with an R<sup>2</sup> value equal to 0.9531(Fig.4).

### 3.3 Comparative analysis of CA15.3 detection by the Au/Cr and Au/ZnO thin films SPR

The intensity changes of two different thin films SPR by adsorbing CA15.3 in the different concentrations (40 · 5 · 0.1U/ml) were shown in Fig.5a. It was indicated that the intensity phase on the Au/ZnO based sensor increased two fold more than that on the Au/Cr layers in 40 U/ml as well as in 5 U/ml. However, under the lower concentration such as 0.1 U/ml, the Au/ZnO based biosensor could improve the sensitivity of detection by 3 times compared to the conventional sensing substrate based biosensor. Moreover,

in the LOD of SPR as shown in Fig.5b, the Au/Cr and Au/ZnO films biosensor reached 0.1 U/ml and 0.025 U/ml, respectively. In other words, the Au/ZnO layers had a detection limit 4 times lower than the Au/Cr layers. This assay was more sensitive than previous reports for tumor marker detection[10, 11]. It can therefore be concluded that an Au/ZnO thin films is a very ultrasensitive sensing substrate for CA15.3 measurement.

#### 4 CONCLUTION

The purpose of this study was to compare the results obtained when using Au/Cr and Au/ZnO based SPR as techniques to measure protein level. The concentration of CA15.3 in healthy individuals is lower than 30 U/mL, indicating that the overall sensitivities of the two different kinds thin films based SPR are adequate to detect this level. We propose the Au/ZnO based thin films to improve the sensitivity with good linearity. These results suggest that the Au/ZnO based SPR biosensor can act as a very good alternative to the Au/Cr based conventional SPR immunosensor.

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(a)	Au	50 nm
	Cr	2 nm
	Glass (n=1.72)	700 μm

(b)	Au	50 nm
	ZnO	50 nm
	Glass (n=1.72)	700 μm

Fig.1. Schematic diagram of the thin films structure (not drawn to scale) in (A) Au/Cr and (B) Au/ZnO

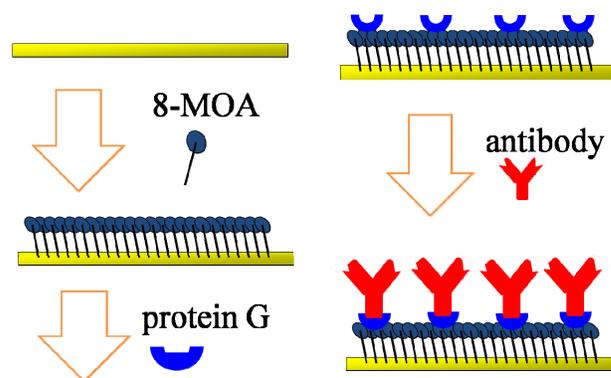


Fig.2. Schematic representations of antibody immobilization on a gold surface

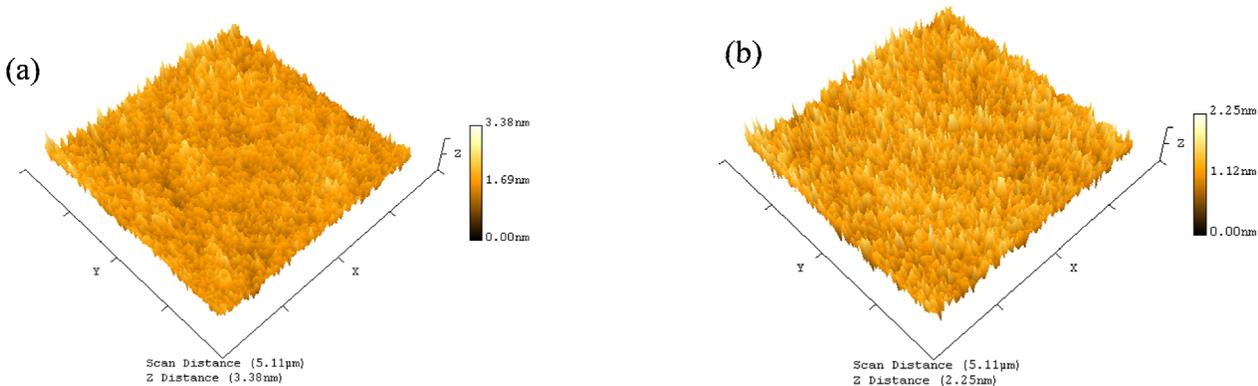


Fig. 3 Topography changes of AFM images on (a) the bare Au/Cr films and (b) the bare Au/ZnO films and the profile measurement of (c) the bare Au/Cr films and (d) the bare Au/ZnO films.

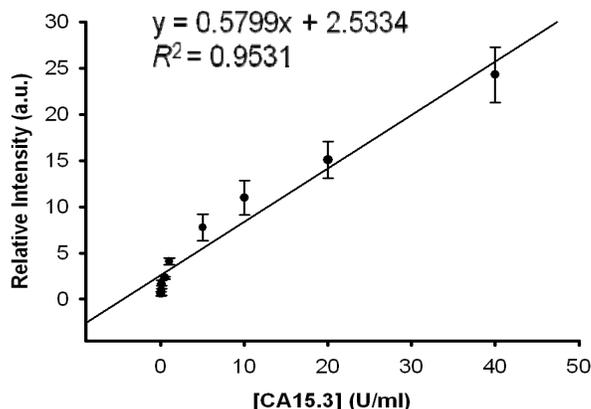


Fig.4 Detection of CA15.3 in pleural fluid using monoclonal antibodies immobilize on the sensor chip. Error bars indicate the standard deviation for the mean of three measurements.

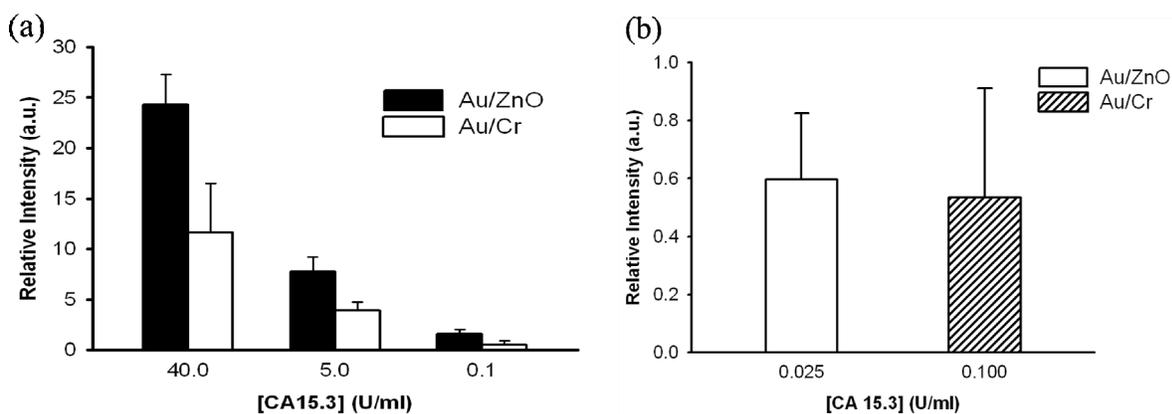


Fig.5 Averages of SPR intensity measure for the adsorption of antigen to antibody (a) at the different concentration and (b) at the detection limit of concentration on the Au/ZnO layers and the Au/Cr layers. Error bars indicate the standard deviation for the mean of three measurements.