# SENSITIVE AND SELECTIVE DETECTION OF A CANCER BIOMARKER, THYROGLOBULIN, IN UNDILUTED HUMAN SERUM USING COMPETITIVE ADSORPTION OF PROTEINS AND PATTERN RECOGNITION

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## ABSTRACT

We report a microfluidic-based Surface Plasmon Resonance (SPR) biosensor for rapid, real-time detection of Thyroglobulin (Tg, a sensitive indicator of persistent or recurrent differentiated thyroid cancer with abnormal concentration higher than 3 ng/mL in undiluted human serum, using competitive adsorption of proteins and pattern recognition. The SPR biosensor produces unique patterns from multiple engineered sensing surfaces, and then Linear Discriminant Analysis (LDA) is used to differentiate these patterns. The microfluidic-based biosensor demonstrates the ability to detect 2 ng/mL Tg in undiluted serum with 91.7 % and 90.0 % classification confidence level for its sensitivity and selectivity, respectively.

## INTRODUCTION

Biomarker proteins are often used to assess the progress of diseases and monitor the effects of the treatment as the relative and absolute levels of them are directly associated with specific disease states. Conventional biosensors rely on specific binding interactions between bio-receptors (i.e., antibody, enzyme, aptamer, etc.) and biomarkers. Unlike these biosensors, our work does not require bio-receptors, and instead utilizes competitive adsorption of proteins [1]. Nevertheless, our previously reported SPR biosensor still relied on specific binding interactions to detect a target biomarker in a controlled cocktail [1]. Such specific binding interactions that can be understood as the "lock and key" analogy [2] often do not provide adequate selectivity in a complex mixture, such as undiluted human serum, partially due to non-specific interferences and weak binding affinity to analyte. To mitigate this challenge, we adopt pattern recognition that relies on cross-responsive interactions [3], rather than a specific interaction. A biosensor array is required to generate a unique composite pattern [4]. For all the bio-receptors in the array, the specific adsorption is not necessary. If only they have different interacting characteristics, the very unique pattern can be generated for discrimination. Without relying on the specific adsorption, the interference from other proteins can be minimized.

In this work, we, for the first time, demonstrate the ability to detect a cancer biomarker with ng/mL sensitivity in undiluted serum using competitive adsorption of proteins and pattern recognition. These cross-responsive interactions are dominated by competitive adsorptions of proteins, namely Vroman effect [5], monitored by an optical-based transducer, SPR (Fig. 1). SPR can very sensitively response to minute changes of refractive index occurring adjacent to a metal film, which may results from the bio-interactions on the sensing surface. A microfluidic module is mounted on SPR to enclose the gold sensing surfaces in microfluidic channels where the competitive adsorption of proteins occurs [6], which produces unique SPR sensorgram. The weak-affinity proteins initially covering the surface are displaced by strong-affinity proteins [5], yet the reverse sequence does not occur. In Fig. 1, two known affinity proteins (Protein 1 & 2) are pre-adsorbed on a bare gold and a COOH-SAM (Self Assembled Monolayer) modified surfaces via the microfluidic channels. After a stable baseline establishing, a Tg-spiked serum sample is injected into the microfluidic channels and flows across the pre-adsorbed surfaces. The SPR angle shift increases simultaneously as weak-affinity pre-adsorbed proteins are replaced by Tg and other strong-affinity proteins in the sample via the competitive adsorption behavior of proteins. Subtracting the baseline, a final angle shift predominantly caused by the competitive adsorption behavior is obtained. The unique surface interactions generate patterns of real-time SPR angle shifts, which then are statistically processed to discriminate patterns using LDA.

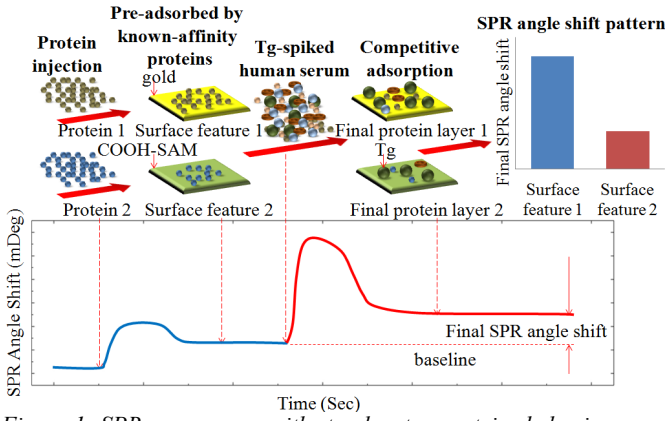


*Figure 1: SPR sensorgram with step-by-step proteins behaviors on a sensing surface based on the competitive adsorption of proteins (Vroman effect).*

## RESULTS

Sensor surfaces are pre-adsorbed by five known-affinity proteins on bare gold and COOH-SAM to form 10 surface features (Table 1). The 10 surface features generate 10 angle shifts, and such process is replicated 6 times. 10 average values of angle shifts form a distinguishing pattern to represent a Tg-spiked sample (Fig. 2(a)). Through LDA, the patterns consisting of different samples can be separated into unique groups of data. Each data point represents a replication generated from the 10 surface features (Fig. 2(b)). As evaluating sensitivity characteristics of our SPR biosensor, 100, 10, and 2 ng/mL Tg-spiked serums are discriminated from each other and the control. Through sensitivity characteristics analysis by LDA, original classification result, 91.7 %, indicates how well the classification functions predict these samples, while cross-validations result, 66.7 %, estimates how well the classification functions derived on given samples predict a subsequent sample.

*Table 1: Matrix of ten sensing surface features; 5 different pre-adsorbed proteins on 2 different surfaces.*

| | Pre-adsorbed proteins | | | | |
|---|---|---|---|---|---|
| | Transferrin | Lysozyme | Albumin | IgM | IgG |
| Bare gold | SF 1 | SF 2 | SF 3 | SF 4 | SF 5 |
| SAM | SF 6 | SF 7 | SF 8 | SF 9 | SF 10 |

*SF: Surface Feature*

*Table 2: Matrix of five samples; control (human serum), and 3 samples of Tg-spiked and one of IgM-spiked serum.*

| Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 |
|---|---|---|---|---|
| Control (human serum) | 100 ng/mL Tg-spiked | 10 ng/mL Tg-spiked | 2 ng/mL Tg-spiked | IgM-spiked |

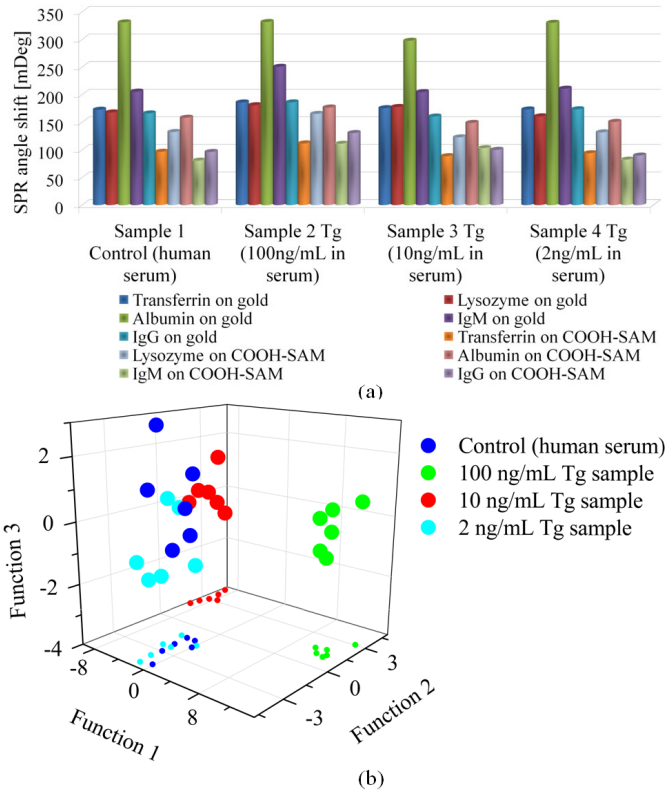


*Figure 2: Sensitivity characteristics: pattern recognition analysis (a) patterns of 4 samples generated from 10 surface features (b) statistical results obtained from LDA (Linear Discriminant Analysis).*

We also analyzed the respective discrimination confidence levels for the three Tg-spiked samples (Table 2). 100, 10, and 2 ng/mL Tg-spiked samples and the control show the original classification of 100 %, 100 %, and 100 %, and the cross-validation of 83.3 %, 53.3 %, and 41.7%, respectively. In other words, for an unknown sample, higher Tg-spiked concentration provides higher possibility to discriminate the Tg sample from the control, which is shown in Figure 2(b). Data points of 100 and 10 ng/mL Tg-spiked samples are far away from those of the control, yet 2 ng/mL Tg-spiked sample has a small but finite overlap with the control, showing the difficulty of discrimination. Obviously higher concentration Tg samples and the control result in more distinctively different patterns that are easier for LDA to discriminate.

For selectivity, IgM-spiked serum sample is discriminated from 100, 10, and 2 ng/mL Tg-spiked serum samples and the control (Fig. 3). IgM is chosen as the affinity strength of IgM is similar to that of Tg. These 5 samples (Table 2) are injected into the microfluidic system, and then flow across 8 surface features. Selectivity characteristics analysis demonstrates 90.0 % original classification, and 63.3 % cross-validations confidence level.

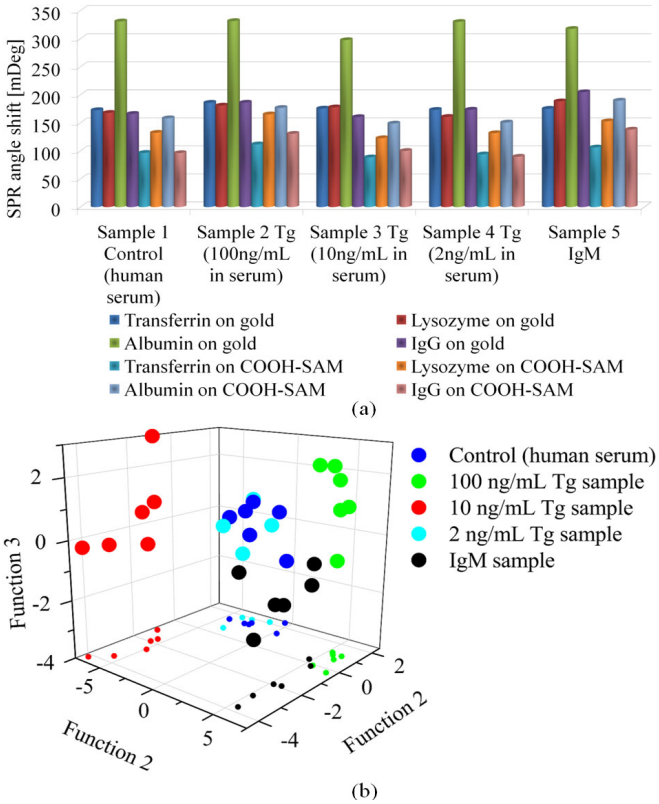


*Figure 3: Selectivity characteristics: pattern recognition analysis (a) patterns of 5 samples generated from 8 surface features (b) statistical results obtained from LDA.*

## ACKNOWLEDGEMENTS

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