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**Development of a Sensitive Detection method of Cancer Biomarkers in Human Serum (75%) using a Quartz Crystal Microbalance Sensor and Nanoparticles amplification system**

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## Abstract

A simple and sensitive sensor method for cancer biomarkers [prostate specific antigen (PSA) and PSA-alpha 1 antichymotrypsin (ACT) complex] analysis was developed, to be applied directly with human serum (75%) by using antibody modified quartz crystal microbalance sensor and nanoparticles amplification system. A QCM sensor chip consisting of two sensing array enabling the measurement of an active and control binding events simultaneously on the sensor surface was used in this work. The performance of the assay and the sensor was first optimised and characterised in pure buffer conditions before applying to serum samples. Extensive interference to the QCM signal was observed upon the analysis of serum. Different buffer systems were then formulated and tested for the reduction of the non-specific binding of sera proteins on the sensor surface. A PBS buffer containing  $200 \mu\text{g mL}^{-1}$  BSA,  $0.5 \text{ M NaCl}$ ,  $500 \mu\text{g mL}^{-1}$  dextran and  $0.5\%$  Tween 20, was then selected which eliminated the interfering signal by  $98\%$  and enabled the biomarker detection assay to be performed in  $75\%$  human serum. By using Au nanoparticles to enhance the QCM sensor signal, a limit of detection of  $0.29 \text{ ng mL}^{-1}$  PSA and PSA-ACT complex (in  $75\%$  serum) with a linear dynamic detection range up to  $150 \text{ ng mL}^{-1}$  was obtained. With the achieved detection limit in serum samples, the developed QCM assay show a promising technology for cancer biomarker analysis in patient samples.

**Keywords:** Cancer biomarkers, prostate specific antigen, PSA, human serum, biosensor, quartz crystal microbalance, QCM, nanoparticles, point of care assay.

## 1 Introduction

The World Health Organisation (WHO) states that around 13% of all deaths worldwide in 2007 were caused by cancer [1]. Early and accurate detection of cancer is very important before it spreads out to the other organs of the body and this makes early diagnosis very important for successful treatment of the disease. The detection of cancer biomarkers in patient samples provides an effective way to diagnose and treat the disease. Recent advances in the area of sensor technology and microarrays have enabled the miniaturisation of the devices and multiplex testing of a range of analytes. Therefore, biosensor technology has the potential to produce point of care cancer testing devices that detects biomarkers [2].

Among different cancer types, prostate cancer which is a complex and multifactorial disease is the commonest form of cancer in men in Europe (301,500 incident cases, 24.1% of all cancer cases) [3]. The increase in prostate specific antigen (PSA) levels in serum above the normal limits is the primary indication of prostate malignancy; therefore PSA is used as a biomarker for the diagnosis and prognosis of the prostate cancer [4; 5]. Several biosensor systems have been applied in the past for the detection of PSA; electrochemical [6; 7; 8], optical [9; 10; 11], fluorescence / chemiluminescence [12; 13], microcantilever [14; 15] and quartz crystal microbalance (QCM) biosensors [16; 17] were used for the detection. The detection limit of PSA using these systems varies between 0.2 pg mL<sup>-1</sup> to 10 ng mL<sup>-1</sup>. The detection signal has been amplified by means of a sandwich assay with Au nanoparticles or the use of carbon nanotubes. In most cases biomarker detection test was performed only in buffered pure solutions rather than serum. To obtain clinically relevant results, it is essential to perform the biomarker test in human serum. The main difficulty of using serum as the assay media is high non-specific interaction between the sensor surface and serum proteins. A number of strategies have been employed to reduce the non-specific binding of clinical samples. As an example Cao *et al.* used mixed self-assembled monolayer coated surface which contains ethylene glycol units [9], Kurosawa *et al.* used 2-methacryloyloxyethyl phosphorylcholine polymer as blocking agent [18] and Situ *et al.* employed additives in the buffer to lower the non-specific binding of serum proteins [19] to the sensor surface. The above described

methods can either be applied individually or together to reduce the non-specific binding of serum proteins achieving different success.

In this paper we describe the development of a simple and rapid detection method for cancer biomarkers analysis using a quartz crystal microbalance (QCM) biosensor. A QCM consists of a thin quartz wafer sandwiched between a pair of electrodes. The mode of oscillation depends on the cut and geometry of the quartz crystal. Mass addition or loss on the sensor surface results in frequency change and hence measuring molecular interactions that occur on the sensor surface without the need of a label. By employing Au nanoparticles in the assay design, it is possible to increase the mass due to antigen binding and further enhance the sensitivity of the assay [20; 21]. However, the frequency is also affected by viscosity change of the assay media and charge of the interaction molecules [22]. Therefore, care needs to be taken to subtract the controls and extract the real response due to specific molecular interactions. The sensitivity enhancement using nanoparticles has been usually achieved by modifying a secondary antibody to the nanoparticles and performing a sandwich assay.

In this work an investigation was performed to develop and optimise an immunoassay to detect biomarkers in human serum on the QCM sensor chip. To minimise the matrix effect of human serum, the addition of detergent, salt and other additives to the buffer solution was investigated. PSA and PSA-ACT complex were used as the cancer biomarkers for detection and an immunoassay was developed and performed in buffer and in human serum.

## 2 Materials and Methods

PSA, monoclonal PSA detection (cat no: MCA2561) and capture antibodies (cat no: MCA2560) were obtained from AbD Serotec, (Kidlington, UK). ACT-PSA complex was purchased from BiosPacific (CA, USA). Mouse IgG (cat no: 015-000-003) usually used as a control antibody was obtained from Stratech Scientific Ltd./Jackson ImmunoResearch (Newmarket, UK). Human serum and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (Poole, UK). All other chemicals were of analytical grade.

## 2.1 Instrumentation

A fully automated QCMA-1 biosensor instrument and sensor chips were obtained from Sierra Sensors GmbH (Hamburg, Germany). Au coated QCMA-1 sensor chips (20 MHz) possess two sensing arrays each, enabling the measurement of active and control sensor surfaces simultaneously (Figure 1S<sup>1</sup>). The operating temperature of the assays was 25°C and the flow rate of the buffer was 80  $\mu\text{l min}^{-1}$  throughout the assay. The data presented in this work are the averages of 4 data points for the assays described unless otherwise stated.

## 2.2 Modification of Au nanoparticles with Anti-PSA detection antibody

PSA detection antibody was added to the 40 nm Au nanoparticle solution and incubated at room temperature on a shaker. BSA was added to the solution to ensure that the Au nanoparticles are all coated with antibody or protein. After spinning 20 minutes at 4°C, the antibody modified nanoparticles were recovered and re-suspended in PBS/T buffer. The concentration of nanoparticles was determined by a spectrophotometer at 520 nm wavelength. The antibody modified Au nanoparticles were then stored in the fridge (4°C) until use.

## 2.3 Sensor surface modification

Initially gold coated QCMA-1 sensor chips were coated with self assembled monolayer (SAM) by immersing the sensors in 2 mM ethanol solution of mercaptoundecanoic acid overnight followed by rinsing with ethanol and water and then dried under nitrogen. The sensors were stored at 4°C till use. For the AFM (Atomic Force Microscopy) study, Dimension 3000 SPM instrument (Veeco Instruments Ltd., Cambridge) was used with silicon probes (type: PPP-NCH-50). The analysis was carried out at tapping mode. AFM images of QCMA-1 sensor were taken before and after surface chemistry application. For the assay, the SAM coated sensor chip was first docked to the instrument and primed with running buffer (10 mM PBS, pH 7.4) at a flow rate of 80  $\mu\text{l min}^{-1}$  until use. Monoclonal anti-PSA antibodies (capture antibody) and Mouse IgG antibody (control antibody) were then immobilised on the sample and control sensing arrays respectively using conventional amine coupling chemistry. Sensor surfaces were first activated with a

<sup>1</sup> Figure numbers ended with letter 'S' are presented at the supplementary information.

1 : 1 mixture of 400 mM EDC and 100 mM NHS by injecting simultaneously across the two sensing spots for 3 min (240  $\mu$ l). A 15-30 or 50  $\mu$ g mL<sup>-1</sup> anti-PSA antibody (in sodium acetate buffer, 10 mM, pH 5.5) was injected on the active sensor array and 15-30 or 50  $\mu$ g mL<sup>-1</sup> mouse IgG (in sodium acetate buffer, pH 5.5) was injected on the control sensor array for 3 min (240  $\mu$ l). The surfaces were then blocked with 50  $\mu$ g mL<sup>-1</sup> BSA in PBS for 3 min (240  $\mu$ l). Non-reacted NHS esters were capped with 1 M ethanolamine, pH 8.5 for 3 min (240  $\mu$ l). The frequency changes were recorded 2 min after the protein injection was completed and the signal reached steady state. The running buffer was changed to PBS containing 0.005% Tween (PBS/T) for the binding assay studies.

#### 2.4 Detection of PSA

In serum, PSA is found either in free form or as a complex with alpha 1-antichymotrypsin (ACT) (ACT-PSA, MW 96 kDA). Total PSA (tPSA) refers to the PSA in both forms (PSA and ACT-PSA complex). In the current study to prepare tPSA 1 to 1 mixture of PSA and ACT-PSA was used. PSA or tPSA was diluted at specified concentrations (0.29 to 5000 ng mL<sup>-1</sup>) in PBS/T buffer containing 5  $\mu$ g mL<sup>-1</sup> BSA or 10% to 75% human serum in PBS/T buffer containing additives. These solutions were then injected over the PSA capture antibody and mouse IgG immobilised surfaces for 3 or 5 min to allow binding interactions (240 or 400  $\mu$ l). The frequency changes due to PSA/tPSA binding were recorded at 180 s after the injection started. After the binding of PSA/tPSA either surface was regenerated by injection of 100 mM HCl (1 min, 80  $\mu$ l) or the assay was continued to perform a sandwich assay.

After the binding of PSA/tPSA to the sensor surface, 1.5  $\mu$ g mL<sup>-1</sup> PSA detection antibodies or PSA detection antibody modified Au nanoparticles were injected on the sensor surface for 3 or 5 min (240 or 400  $\mu$ l). After 3 min dissociation period under running buffer flow, surfaces were regenerated by injection of 100 mM HCl (1 min, 80  $\mu$ l) (and additional 20 mM NaOH (1 min, 80  $\mu$ l) was injected if PSA spiked human serum was used). The frequency changes due to PSA detection antibody binding were recorded 3 min after the injection started. The limit of detection (LOD) was calculated as the signal obtained from the PSA concentration that is equivalent to the 3 times the standard deviation of the signals obtained from the blank standards.

### 3 Results and Discussion

#### 3.1 Optimisation of sensor signal

Bare gold QCM-A-1 sensor chips were employed in this work as the sensor platform for PSA detection. Each chip consists of two sensing array for active and control sample testing. The modification of the chips using self assembled monolayer (SAM) was carried out on the sensor surface. Atomic Force Microscopy (AFM) images of a sensor chip were taken before and after the SAM coating (Figure 2S). The results indicate that SAM modification caused a change of the root mean square (rms) roughness from 5.5 nm to 3.1 nm due to surface coating. The SAM coated sensor chip was first docked to the QCM-A-1 instrument and primed with running buffer to wet the sensor chip and continues buffer flow ( $80 \mu\text{l min}^{-1}$ ) was started. Anti-PSA capture antibody and mouse IgG were then immobilised on the active sensor array and the control sensor array respectively using conventional EDC-NHS chemistry [23]. A 3 minutes injection of antibodies was sufficient to achieve signal saturation; therefore, the immobilisation time was kept at 3 minutes for the assay. Three different concentrations were used for antibody immobilisation and from these  $30 \mu\text{g mL}^{-1}$  was chosen as the optimum concentration to immobilise the antibodies. Anti-PSA capture antibody immobilised sensors produced an average frequency change of  $380 \pm 38 \text{ Hz}$  and mouse IgG immobilised sensors produced a frequency change of  $520 \pm 15 \text{ Hz}$  (Figure 3S).

The PSA non-specific binding (diluted in PBS/T buffer containing  $5 \mu\text{g mL}^{-1}$  BSA) to the control sensor array was then examined by conducting binding tests. The results show that the control sensor surface did not give any frequency change even if the highest concentration of PSA ( $5 \mu\text{g mL}^{-1}$ ) was used (Figure 1-trace e). To examine the non-specific binding of BSA on the PSA capture antibody immobilised surface, BSA ( $5 \mu\text{g mL}^{-1}$ ) was injected for 3 min and the non-specific binding of BSA to the surface was detected as  $4 \pm 1 \text{ Hz}$  ( $n = 3$ , data not shown).

For the sandwich assay procedure, anti-PSA detection antibody ( $3 \mu\text{g mL}^{-1}$ ) was injected in the absence of the antigen (PSA) on to the PSA capture antibody immobilised surface

and the non-specific binding was detected as  $5 \pm 2$  Hz ( $n = 3$ , data not shown). The non-specific responses were subtracted from the PSA or PSA detection antibody binding data.

### 3.2 Determination of kinetic constants for PSA detection assay

The calibration curve obtained with PSA binding (3 min) to the PSA capture antibody immobilised surface in a concentration range between 4.7 to 5000 ng mL<sup>-1</sup> is shown in Figure 1 and Figure 4S. PSA binding response curves were then fitted to 1:1 Langmuir binding model to determine the binding association and dissociation rates [24], from which  $K_D$  value was calculated as  $5.56 \times 10^{-10}$  M and  $R_{max}$  as 108 Hz (Table 1). Karlsson *et al.* calculated the affinity of PSA antibody towards PSA as  $3.3 \times 10^{-9}$  M (antibody from Fitzgerald Industries Int., clone M212091) [25]. Katsamba *et al.* calculated the affinity as  $1.1 \times 10^{-9}$  M (antibody from Fitzgerald Industries Int., clone M612166) [26].

To enhance the sensor signal and improve the sensitivity of the method, a sandwich assay approach was followed employing PSA detection antibody. The calibration curve was obtained for the sandwich assay in a concentration range between 150 to 2.3 ng mL<sup>-1</sup> that is relevant for prostate cancer diagnosis (Figure 4S, trace b). This assay resulted in 4.7 ng mL<sup>-1</sup> PSA as detection limit which is four folds more sensitive than the direct assay (Table 2). The experiments have shown that the chosen anti- PSA antibody has good affinity for a successful immunoassay and the sandwich assay resulted in a clinically relevant detection limit. Consequently experiments were performed to optimise the assay so that biomarker detection could be performed in human serum.

### 3.3 Buffer optimisation for human serum sample analysis

There is a significant difference in the limit of detection when buffer or clinical sample (serum) is used as the assay media. For example, although Cao *et al.* performed the PSA-ACT detection assay using only 10% human serum; the limit of detection has changed from 10.2 ng mL<sup>-1</sup> (in buffer) to 18.1 ng mL<sup>-1</sup> (in 10% serum) [9]. This change in the detection limit, when clinical samples are used, is mainly due to lower signal to noise ratio resulting from the high non-specific binding of sera proteins/antibodies to the sensor surface. This is especially noticeable when label-free biosensors are employed, and this prevents researchers from the use of high concentrations of human serum. Therefore,

most analyses are conducted using concentration of human serum in the range 10 to 50 % [18; 19; 27].

In the current study we investigated the use of additives to minimize the adsorption of sera proteins to the sensor surface. Initially, a 10% human serum diluted in PBS/T buffer was injected on the mouse IgG immobilised sensor surface. A 1490 Hz response was obtained from this solution and after buffer flow started, the response was reduced to 1370 Hz (Figure 2, A-trace 1). From the results it was evident that human serum proteins caused a significant non-specific binding on the surface. To reduce this non-specific binding salt, BSA, dextran and Tween 20 were added to the buffer at varying concentrations. Increase the salt concentration increases the ionic strength of the solution and that lowers the electrostatic attraction of human serum proteins to the sensor surface. Therefore, high salt concentration was used to reduce proteins adsorption to the sensor surface. Dextran and BSA were used to help in preventing serum protein absorption. It is common to employ detergents to lower the non-specific protein binding; therefore the concentration of Tween 20 was increased in the solution [28]. These additives at varying concentrations were added to prepare 10% human serum and injected over the mouse IgG immobilised sensor surface for 3 min (Figure 2, A). The lowest non-specific binding was observed when the serum is diluted in additives containing PBS/T buffer that had final concentrations of  $200 \mu\text{g mL}^{-1}$  BSA, 0.5 M salt,  $500 \mu\text{g mL}^{-1}$  dextran and 0.5% Tween 20 in solution. The response 180s after the injection of 10% human serum diluted in this matrix elimination buffer (matrix buffer) was found as  $251 \pm 18 \text{ Hz}$  ( $n = 3$ ) and after the buffer flow started the response was reduced to  $23 \pm 12 \text{ Hz}$  ( $n = 3$ ). As it can be seen from Figure 2, A - trace 1, the injection of human serum in PBS/T buffer resulted in a trace that showed a curved structure indicating the binding of sera proteins to the sensor surface and there was little dissociation after the injection stopped and buffer flow was started. Whereas when human serum diluted in the matrix buffer was injected (Figure 2, A - trace 5), the response trace showed an instant sharp increase up to ca. 250 Hz and remained showing no curvature indicated that the bulk of this response was due to the use of additives and therefore this matrix buffer was then used for all further assays using human serum. Figure 2, B, shows the comparison of non-specific binding results for 10%, 40% and 75% human serum diluted in the matrix buffer developed in this work and injected to mouse IgG immobilised surface. The low non-specific binding values for these three concentrations of human serum (highest  $91 \pm 10 \text{ Hz}$  for 75% human serum) indicated that

the matrix buffer was effective even for high serum concentrations. For all the forthcoming experiments human serum was always diluted with the matrix buffer.

These results showed that by applying the matrix buffer in the assay method, a 98% reduction in non-specific binding of human sera proteins was achieved. If this result is compared to other recently published work; Situ et al. achieved 94% reduction in non-specific binding by using CM5 (carboxymethyl dextran) surface and a formulated buffer together and Trevino et al. achieved 88% reduction in non-specific binding of serum by using another formulated buffer [19; 29].

The PSA assay was then performed using 20 ng mL<sup>-1</sup> PSA spiked in 10% and 40% human serum. When the results were compared with PSA assay in PBS/T buffer, it was seen that both direct and sandwich PSA assay results in buffer or spiked in 10% human serum (diluted in matrix buffer) were very close to each other and within the standard deviation (Figure 3, A). However, as the human serum concentration increased to 40%, the response from direct assay was eliminated but still there was a response from the injection of PSA detection antibody (sandwich assay). Although this result was lower than the assay in PBS/T buffer (or assay in 10% human serum) still good enough to perform the assay in high human serum concentrations. An example to direct and sandwich assay response of PSA spiked in 10% human serum is shown in Figure 3B.

### 3.4 Detection of tPSA in 75% human serum

A calibration curve was obtained for the sandwich assay in a concentration range between 2.3 to 150 ng mL<sup>-1</sup> tPSA spiked in 75% human serum. The non-specific binding of PSA detection antibody after the injection of 75% human serum (without spiked PSA) was  $2 \pm 1$  Hz, if assay performed on mouse IgG immobilised surface and  $10 \pm 3$  Hz if assay performed on PSA capture antibody immobilised surface. The PSA detection antibody binding responses were obtained after the subtraction of the non-specific binding response. The detection limit obtained for this assay was 9.4 ng mL<sup>-1</sup> (Figure 5 - trace a). However this result was not sensitive enough for PSA detection at the required levels in serum. To enhance the sensitivity further 40 nm Au nanoparticles were modified with PSA detection antibody and sandwich assay was performed to detect tPSA. The use of Au nanoparticles lowered the detection limit down to 0.29 ng mL<sup>-1</sup> (Figure 5 – trace b), which

is well below the required limit of detection. Table 2, summarises the results of the assays in buffer and in human serum.

The specificity of the tPSA interaction with the sensor surface was tested by employing a mouse IgG surface on the control sensor. The non-specific binding of PSA detection antibody modified Au nanoparticles after the injection of 75% human serum (with or without spiked tPSA) resulted in no response on mouse IgG immobilised surface (Figure 4 - trace b). The binding of PSA detection antibody modified Au nanoparticles after the injection of 75% human serum (without spiked tPSA) resulted in  $9 \pm 2$  Hz response on anti-PSA capture antibody immobilised surface, since non-specific binding on to MIgG surface was very low, this result was purely due to the tPSA content of the serum obtained from Sigma. While creating the calibration curve, this response was deducted from the responses obtained to get the binding results due to the spiked tPSA. Later after calibration curve of tPSA assay was obtained, it was calculated that serum from Sigma used for the study contains  $0.41 \text{ ng ml}^{-1}$  tPSA.

In conclusion, the limit of detection of the biomarker assay was  $0.29 \text{ ng ml}^{-1}$  (corresponds to  $0.39 \text{ ng ml}^{-1}$  in 100% serum) with a linear detection range of  $0.29\text{-}150 \text{ ng ml}^{-1}$  and the coefficient of variation (CV) laid between 1 to 9% across the range. These results show that the assay developed with the formulated matrix buffer and Au nanoparticle signal amplification, have potential for the rapid and sensitive detection of serum biomarkers in patient samples.

The number of publications related to the detection of PSA using QCM biosensor is very limited and this indicates that application of QCM biosensors to PSA detection is a newly developing area. An example to a previous study for PSA assay using QCM biosensor has been the study performed by Zhang *et al.* which involve the use of an insert-plug model of piezoelectric immunosensor. After 40 min of reaction at  $37^\circ\text{C}$  in a static cell with a 10 MHz quartz crystal, Zhang *et al* achieved linear detection of  $1.5 - 40 \text{ ng mL}^{-1}$  PSA in 100% serum [17]. Although the detection limit obtained from this study was low enough, the reaction time was long and assay needs to be performed at elevated temperatures. Additionally the dynamic range of the assay was limited. In another study, Ding *et al.* used yeast cell modified QCM sensors to immobilise PSA antibodies [16]. The binding of PSA to the PSA antibody immobilised sensor was recorded after the binding reached

equilibrium using a 9 MHz quartz crystal and magnetic stirrer agitated static cell. With this method Ding *et al.* achieved detection of PSA in the range of 5 to 604 ng mL<sup>-1</sup> in 100% serum. Both applications mentioned above were performed using non-automated equipment that due to their manual operation may not only cause variations between the assays but also are not suitable for clinicians use. In another study Cao *et al.* performed 10 min PSA-ACT assay in 10 % human serum using an automated SPR biosensor and obtained a detection limit of 18.1 ng mL<sup>-1</sup> that corresponds to LOD of 181 ng mL<sup>-1</sup> for the whole serum [9].

In our study although 75% serum was used as assay matrix, the detection limit obtained was 0.29 ng mL<sup>-1</sup> for a 8 min assay which corresponds to 0.39 ng mL<sup>-1</sup> tPSA in the whole serum indicating the good performance of the optimised assay. Additionally; although high concentration of serum was used for the assay, matrix buffer limited the non-specific binding of the proteins on to sensor spots and allowed complete regeneration of the sensor surface by short injections of 100 mM HCl and 20 mM NaOH. This enables the repeated use of the same sensor chip for several times to analyse multiple serum samples to detect tPSA.

The experimental conditions reported in the literature vary between the assays significantly such as, assays in static or in flow, temperature, assay time and the antibody used against PSA. All these contribute to the assay sensitivity and hence make it difficult to compare the results effectively. When compared briefly to similar label-free PSA assays such as above, the assay developed in this study, with total 8 min reaction time, repeated usability and LOD of 0.29 ng mL<sup>-1</sup> in 75% human serum, proves the advantage of the optimised assay format suitable for point of care system. Additionally due to it is being rapid and wide detection range the QCM assay developed outperforms the commercial ELISA kits available for biomarker detection (Table 3).

#### 4 Conclusion

Early diagnosis of cancer biomarkers is essential for successful treatment of the disease. The current biomarker tests are ELISA type and usually performed at centralised labs using automated devices. In this work, a groundwork study was described for the use of QCM biosensor to perform cancer biomarker detection test. A new buffer was formulated to eliminate 98% of the non-specific human serum protein binding to the sensor surface.

A sandwich assay using QCMA-1 affinity sensor chips resulted in detection of tPSA concentrations as low as  $0.29 \text{ ng mL}^{-1}$  in 75% human serum. Short assay time, repeated usability of the same sensor chip, ability to detect PSA in high serum concentrations and utilisation of a label-free detection method enables the optimised assay format a promising tool for clinical diagnosis/prognosis.

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Figure 4.

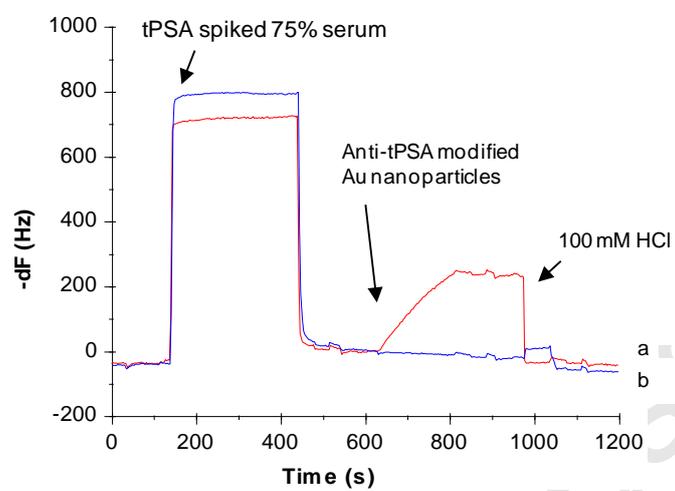


Figure 5.

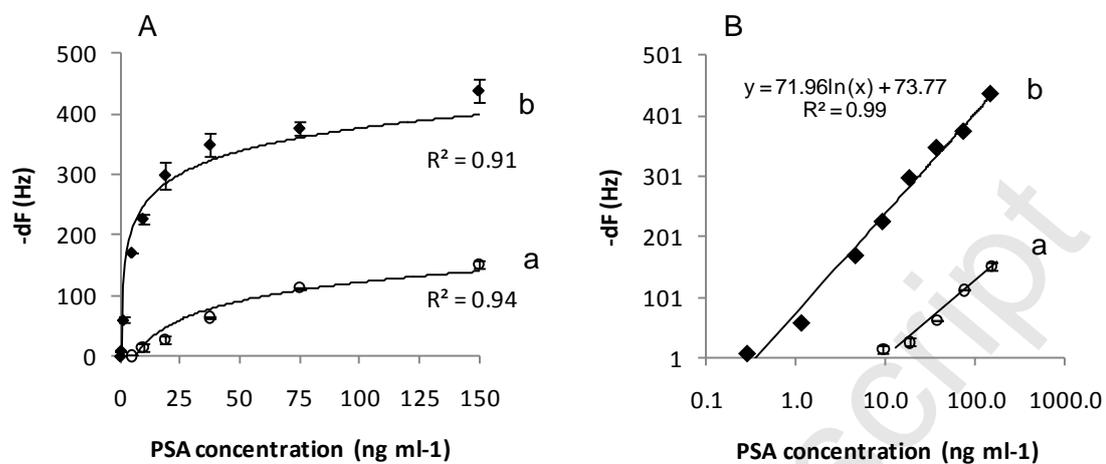


Table 1. Results of kinetic calculations for PSA direct assay.

$K_D = 5.56 \times 10^{-10} \text{ M}$
$K_a = 1.23 \times 10^{+06} \text{ M}^{-1} \text{ s}^{-1}$
$K_d = 6.83 \times 10^{-04} \text{ s}^{-1}$
$R_{\text{max}} = 108 \text{ Hz}$

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Table 2. The summary of the PSA detection assay results.

	<b>direct assay in PBS/T</b>	<b>sandwich assay in PBS/T</b>	<b>sandwich assay in 75% serum</b>	<b>sandwich assay in 75% serum using Au nanoparticles</b>
LOD	18.8 ng mL <sup>-1</sup>	4.7 ng mL <sup>-1</sup>	9.4 ng mL <sup>-1</sup>	0.29 ng mL <sup>-1</sup>
Linear range	18.8 – 150 ng mL <sup>-1</sup>	4.7 – 150 ng mL <sup>-1</sup>	9.4 – 150 ng mL <sup>-1</sup>	0.29 – 150 ng mL <sup>-1</sup>
Equation	$y = 0.87x + 0.27$ ( $R^2 = 1.00$ )*	$y = 0.91x + 0.35$ ( $R^2 = 0.99$ )*	$y = 0.89x + 0.33$ ( $R^2 = 0.98$ )*	$y = 71.96\ln(x)+73.77$ ( $R^2 = 0.99$ )

\*y = log (response); x = log (concentration)

Table 3. The summary of the PSA detection limit and detection ranges for some of the commercial ELISA kits and the developed QCM assay.

	<b>Detection range (ng mL<sup>-1</sup>)</b>	<b>Limit of detection (ng mL<sup>-1</sup>)</b>
The developed QCM assay*	0.39 – 200	0.39
Abazyme PSA ELISA kit	1 - 80	1
MP Biomedicals PSA ELISA kit	2 - 120	1
Alpco Diagnostics PSA ELISA kit	2 - 120	1
Calbiotech PSA ELISA kit	0.585 - 50	0.585
Alpha Diagnostic PSA ELISA kit	0.3-60	0.30

\*Both detection range and limit is given based on 100% serum.