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Research paper

BSA /anti BSA Interaction Study using a Novel Modular Surface Plasmon Resonance (m-SPR) Device

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Abstract

A novel modular surface plasmon resonance (SPR) device fabricated in-house was tested for its function and linearity for biomolecular interaction using BSA and α-BSA as model biomolecules. The sensor surface was initially saturated with BSA molecules and their interactions with α-BSA molecules were then monitored over various concentrations of α-BSA. The newly developed modular SPR biosensor was able to detect biomolecular interactions between BSA and α-BSA at α-BSA with good linearity. Stabilization of the interactions was achieved within 10 minutes of monitoring time.

Keywords: *Surface plasmon resonance; biosensors; portable; biomolecular interaction*

1. Introduction

Surface plasmon resonance (SPR) is a type of optical sensor introduced in the seventies for characterization of thin films and monitoring processes at thin metal interfaces [1,2]. The eighties observed the advanced application of SPR for gas detection and biosensing [3-5]; and since then the technique had popularized among the scientific community for label-free, real time monitoring of biomolecular interactions. Advancement in SPR instrumentation is directed towards an integrated, miniaturized, low cost, portable SPR. More recently, application of SPR for the development of point-of-care devices has been a primary area of focusing SPR technology [6-15].

Commercially available SPR are lab-based, bulky and expensive [16]. Samples for analysis need to be transported from its original source to the lab for analysis. Various sample preparation steps may be required before samples are packaged and shipped for laboratory analysis and during this period of transporting of the samples, reduction and contamination of sample activity may occur, thus affecting the accuracy of the test results [17-20]. For these reasons, miniaturization of the SPR sensor is not only an advantage but crucial to the development of the technology in the medical and health industries.

We have developed a modular SPR system based on the modification of a reflection sampling system (RSS-VA) device from Ocean Optics. The fabrication and testing of the equipment using solvent of various refractive indices were discussed elsewhere [21-22]. This paper discusses the capability of the device to measure biomolecular interactions. Bovine serum albumin (BSA) and anti-bovine serum albumin (α-BSA) were used as model proteins.

2. Materials

2.1. Chemicals and raw materials

Anti-bovine serum albumin (α-BSA, MW 69 kDa produced in rabbit), hydrochloric acid (20 mM, HCl), 11-mercaptoundecanoic acid (MUA), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), N-Hydroxysuccinimide (NHS), were obtained from Sigma Aldrich (St. Louis, USA) and absolute ethanol 99% were purchased from John Kollin Corporation (UK). Phosphate saline buffer, pH 7.4 (PBS) 10 mM was freshly prepared by dissolving sodium chloride (NaCl), potassium chloride (KCl), sodium hydrogen phosphate (Na_2HPO_4) and potassium dihydrogen phosphate (KH_2PO_4) in ultrapure water. All chemicals for PBS solution were purchased from Sigma-Aldrich (St. Louis, USA).

2.2. Modular SPR device

The setup of modular SPR device can be divided into three parts as shown in fig. 1. Part A constitutes the polychromatic light source (LS-LED, Ocean Optics, Inc, USA) and connected to the polarizer via a fibre optic cable (QP450-0.25XSR, Ocean Optics, Inc, USA) to produce *p*-polarized light. Part B is a reflection sampling system (RSS-VA, Ocean Optics, Inc, USA) that have been modified for SPR purposed. It comprises the incident angle control, optical system and the sensing element. The incident angle of light beam is fixed at 29°. A gold coated (50 nm) glass disk is used as a sensor chip and purchased from Metrohm Autolab (Netherlands). Sample is introduced into the flow cell by peristaltic pump and the flow rate was kept at 20 µl/min throughout the experiment.

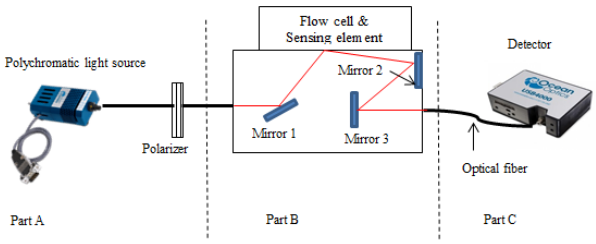

Fig. 1: Schematic diagram of modular SPR sensor system. The system is divided into three parts; part A consists of polychromatic light source and polarizer; part B consists of flow cell, sensing element and peristaltic pum and part C consists of Spectrometer and laptop computer for signal processing. All parts are connected via fiber optics.

Light beam from part A is reflected by mirror-1 and strikes the sensing element to excite surface plasmon on the sensor chip and the reflected light beam is guided by mirror-2 and mirror-3 towards the part C which is detector (USB 4000, Ocean Optics, Dunedin, USA) for data analysis. Light source is allowed to warm up for 10 minutes to ensure the stability of the light intensity output. During all the experiment, the USB 4000 spectrometer integration time is set to 1 second, scans to average to 1 and boxcar width to 8.

2.3 Sample preparation

Stock solutions of BSA and α-BSA were equilibrated at room temperature before dilution to ensure maximum activation of the biomolecules. Five series of ligand (BSA) concentration were prepared by dissolving 2 mg/ml stock solution of BSA into the PBS solution. The concentrations of prepared ligand were 8, 16, 32, 64 and 128 µg/ml. Stock solution of analyte (α-BSA) concentration of 2.4 mg/ml were dissolved in PBS and divided into various concentrations of 12, 24, 48,86 and 192 µg/ml. PBS concentration of 10 mM is used as a blank sample. All biological samples were aliquot and stored at -20°C prior to use

2.4. Immobilization of BSA and saturation of sensor surface

The sensor surface was cleaned in a mixture of concentrated sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2) at a ratio of 3:1. The cleaned sensor chip was then immersed in 2 mM MUA solution overnight and rinsed with ultrapure water 5 times to remove excess thiols on the sensor surface. The sensor chip was placed on the sensing region and a mixture of EDC/NHS (1:1) was allowed to flow across the sensor surface for 8 minutes to activate the self-assembled monolayer of MUA molecules. Then, various concentrations of BSA (8, 16, 32, 64 and 128 µg/ml) were introduced into the flow cell at a flow rate of 20 µl/min for 8 minutes to determine the minimum concentration of BSA required for saturation of sensor surface. For the activation of SAM on the sensor surface, EDC/NHS mixture must be freshly prepared.

2.5. Bio-sensing protocol for interaction of BSA and anti-BSA

The steps involved in the experiment for studying interaction between BSA and α-BSA is summarized as follows:

1. PBS was introduced onto the modified sensor surface to rinse the sensor surface and remove air bubbles along the Teflon tube for 5 minutes at a flow rate of 20 µl/min.
2. A predetermined concentration of BSA (identified from section 2.3.2) was introduced onto the sensor surface for 8 minutes.
3. PBS was introduced onto the sensor surface as a blank sample for 8 minutes at a flow rate 20 µl/min and the resonance wavelength after stabilization was recorded.
4. α-BSA solutions (12 µg/ml) were then introduced into the flow cell and allowed to bind to the surface immobilized BSA for 8 minutes at the same flow rate as above. The resonance wavelength after stabilization was recorded.
5. Then, PBS was introduced onto the sensor surface followed by 20 mM HCl (regeneration solution) for 4 minutes.
6. Step 4 and 5 were repeated for other concentrations of α-BSA
7. The same sample is repeated for 5 times to get the average and standard deviation.

3. Result and Discussion

3.1. Immobilization of BSA and saturation of sensor surface

The gold surface is modified with the MUA solution to obtained the carboxylic group chain (-COOH) on the sensor surface. This carboxylic group was covalently couple with the sulphur (-S) group on the sensor surface and BSA was covalently coupled to the carboxyl group of MUA sensor surface through amide bonds formed between the carboxyl groups in MUA with amino group in the protein. A schematic diagram of the interaction is shown in fig. 2.

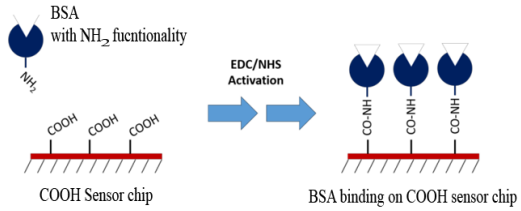

Fig. 2: Schematic diagram of interaction of ligand (BSA) with modified sensor surface. BSA is bound onto the sensor surface via covalent bonding through amide bonds.

Fig. 3 shows the signal shifts upon immobilization of BSA of various concentrations. The resonance wavelength obtained for BSA concentration of 8, 6, 32, 64 and 128 µg/ml are 591, 592, 594, 598 and 598 nm respectively. The signal increased linearly with BSA concentration until the addition of 64 µg/ml of BSA; after which it started to level off. The minimum amount of BSA molecules required to saturate the sensor surface was therefore identified as 64 µg/ml and the resonance wavelength obtained is 598 nm. At this concentration the sensor surface have full coverage of the BSA molecules. Incomplete coverage of sensor surface could lead to nonspecific binding and false measurement of biomolecular interactions. This BSA concentration was used to study biomolecular interactions between BSA and α-BSA molecules in the next section.

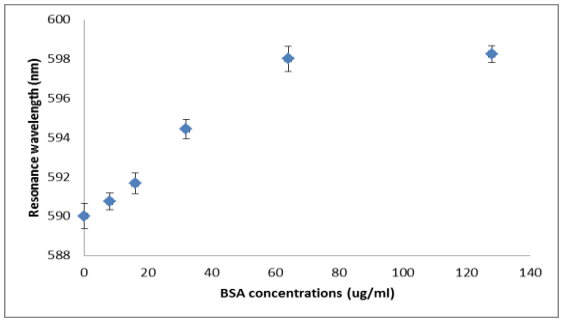

Fig. 3: The signal shifts (resonance wavelength) upon immobilization of BSA of concentrations (8-128) µg/ml.

3.2. BSA/α-BSA biomolecular interaction

After the immobilization of the BSA onto the sensor surface onto the sensor surface, PBS solution was introduce onto the sensor surface for 8 minutes to check the stability of the BSA-bound-sensor chip on the sensor surface. There is no shift in the sensor signal indicated the BSA was strongly bound on the sensor surface. This PBS solution is act as the baseline signal and the resonance wavelength obtained is 598 ± 0.1 nm. The sensor signal shifted instantaneously when 12 µg/ml α-BSA was introduced onto the sensor surface and stabilized after about 8 minutes until the saturated signal is obtained. The signal is shifted from 598 ± 0.1 into 600 ± 0.1 nm.

Before the addition of new sample, regeneration solution (20 mM HCl) is introduced onto the sensor surface. The purposed of regeneration solution is to remove α-BSA that was bound with the BSA on the sensor surface. The surface then rinsed with PBS solution and the sensor signal is back to the baseline signal (598 ± 0.1 nm). This indicated that the regeneration solution not destroys the BSA layer that was bound on the sensor surface and the sensor surface can be used for measuring the next sample. The sample of α-BSA solution with the concentration of 24, 48, 96, and 192 µg/ml was introduced onto the sensor surface and between the additions of a new sample, regeneration solution must be flow first to remove a previous α-BSA sample followed by rinsed with PBS to make sure the signal is back to the baseline signal. This indicates the signal have a good repeatability because of the baseline signal is not shifting during the measurement of each sample.

The temperature of the liquid sample may disturb the accuracy of the SPR signal. The ambient temperature of the each liquid samples and cell is measured every 8 minutes to make sure there are no temperature drift occurred during the experiments. The temperature is maintained at 24 ± 1.0 °C. The cycle of each sample measurement is shown in fig. 4.

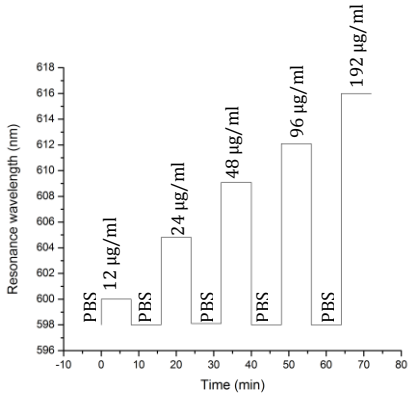

Fig. 4: The signal shifts (resonance wavelength) upon immobilization of α-BSA onto the sensor surface (12-192 µg/ml)

After BSA was immobilized onto the sensor surface, a sample solution with a selected concentration of α-BSA was introduced onto the sensor surface for 8 minutes. This resulted in a shifted in resonance wavelength towards the longer wavelength. This is because the refractive index in the vicinity of the sensor surface is increased due to the interaction of BSA and α-BSA. Basically, increasing the concentration of the α-BSA will increase the binding sites between BSA and α-BSA, thus increasing the refractive index in vicinity of the sensor surface.

Fig. 5 shows the raw data for each sample. The raw data obtained from the SpectraSuite software (Ocean Optics, Dunedin, USA) and further processed using OriginPro 8 software (OriginLab, Northampton, MA). The raw data obtained for each sample were filtered with a Savitzky-Golay filter and the resonance wavelength of sample is determined using the "minimum hunt method" [23]. In this method, a second order polynomial is fit to the SPR dip spectrum to determine the local minimum which is the resonance wavelength.

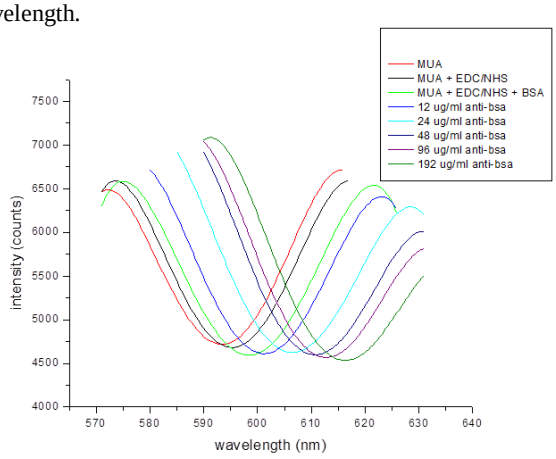

Fig. 5: Resonance wavelength of various concentrations of analyte. Shift in resonance wavelength is equilibrium after 8 minutes of the flow of the analyte solutions at the flow rate 20 µl/min.

The resonance wavelength correspond to the each sample is shown in fig. 6. The resonance wavelength obtained for 12, 24, 48, 96, 192 µg/ml are 600, 605, 610, 612 and 616 ± 0.1 nm respectively.

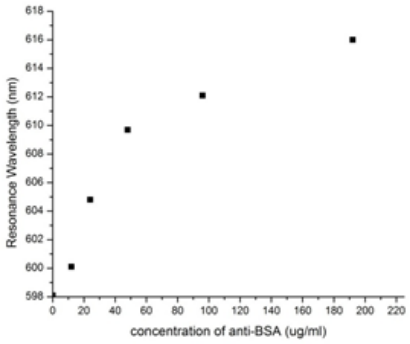

Fig. 6: Resonance wavelength of various concentrations of analyte. Shift in resonance wavelength is equilibrium after 8 minutes of the flow of the analyte solutions at the flow rate 20 µl/min.

From fig. 6, the linearity behaviour of the resonance wavelength was obtained only from the concentration of α-BSA from 12 – 48 µg/ml. The sensor lost the linearity behaviour when the sample of concentration of 96 µg/ml was introduced onto the sample surface. In order to determine the sensitivity and the limit of detection of the sensor, the resonance wavelength shift correspond to the α-BSA concentration of 12, 24, 48 is plotted. The linearity of sensor response is indicated in fig. 7.

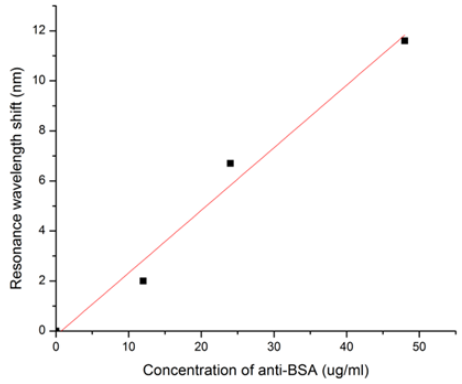

Fig. 7: Linearity of resonance wavelength shift for three concentration of α-BSA (12, 24 and 48 µg/ml). Value of R^2 is 0.98 (b).

The resonance wavelength shift is obtained by the subtraction of the resonance wavelength of sample with the resonance wavelength of blank sample (PBS) as expressed in equation 1.

$$\Delta\lambda = \lambda_{sample} - \lambda_{blank} \quad (1)$$

The signal shifted obtained for α-BSA concentration of 12, 24 and 48 µg/ml are 4, 6, 11 ± 0.1 nm respectively. The sensitivity of the sensor can be expressed in equation 2.

$$\text{Sensitivity, } S = \Delta\lambda / \Delta c \quad (2)$$

Where Δλ is the different in the resonance wavelength and Δc is the different in the concentration of the sample. From the linear graph in fig. 7, the gradient of the graph is corresponding to the sensitivity of the sensor and it was found to be 0.1275 nm / µg ml^{-1}. It means that the resonance wavelength will be shit to 0.1 nm for 1 µg/ml of α-BSA concentration.
Theoretically, based on the extrapolation of data in fig. 7, the concentration as low as 8 µg/ ml could be detected by the modular SPR device. This estimation is based on the calculation of limit of detection based on linear regression analytical method [24]. The performance of the sensor is comparable with these papers [25, 26]. In principle, the sensor's detection limits may be further improved by using the high resolution detector and using a secondary antibody, which can also validate the analyte and improve specificity of the detection.

4. Conclusion

The presented modular SPR biosensor shows the capability of detection between BSA and α-BSA at concentrations of 12 to 192 µg/ml. The sensor signal shifted instantaneously and saturated in about 8 minutes and showed good repeatability. The linearity of the sensor surface was found for the α-BSA concentration in the range of 12 – 48 µg/ml. Based on regression analytical method, the detection limit of the sensor for α-BSA was found to be as low as 8 µg/ml. The sensor performance could be improved by upgrading all modular parts to the latest specification such as using a high resolution detector.

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References

[1] E. Kretschmann and H. Raether (1968), Radiative decay of non-radiative surface plasmons excited by light. *Zeithschrift für Naturforschung* 23, 2135–2136.
[2] I. Pockrand, J. D. Swalen, J. G. Gordon, and M. R. Philpott (1978), Surface plasmon spectroscopy of organic monolayer assemblies. *Surface Science.* 74, 237–244.
[3] B. O. Liedberg (1983), Surface plasmon resonance biosensing* for gas detection and. *Sensors and actuators* 4, 299–304.
[4] C. R. Lowe (1985), An introduction to the concepts and technology of biosensors. *Biosensors* 1, 3–16.
[5] B. Rothenhäusler and W. Knoll (1988), Surface–plasmon microscopy. *Nature* 332, 615.
[6] W. Na, M. Kang, D. Jang, and S. Shin (2018), Miniaturized surface plasmon resonance biosensor with vacuum-driven hydrodynamic focusing. *Sensors and Actuator B: Chemical* 254, 64–71.
[7] B. A. Prabowo, L.-C. Su, Y.-F. Chang, H.-C. Lai, N.-F. Chiu, and K.-C. Liu (2016), Performance of white organic light-emitting diode for portable optical biosensor. *Sensors Actuators B Chemical* 222, 1058–1065.
[8] B. Derkus (2016), Applying the miniaturization technologies for biosensor design. *Biosensor and Bioelectronic* 79, 901–913.
[9] Y. Liu, Q. Liu, S. Chen, F. Cheng, H. Wang, and W. Peng (2015), Surface plasmon resonance biosensor based on smart phone platforms. *Scientific Reports* 5, 1–9.
[10] S. S. Zhao, N. Bukar, J. L. Toulouse, D. Pelechacz, R. Robitaille, J. N. Pelletier, and J. F. Masson (2015), Miniature multi-channel SPR instrument for methotrexate monitoring in clinical samples. *Biosensors and Bioelectronic* 64, 664–670.
[11] I. Arghir, F. Delport, D. Spasic, and J. Lammertyn (2015), Smart design of fiber optic surfaces for improved plasmonic biosensing. *New Biotechnology* 32, 5, 473–484.
[12] R. Chen, M. Wang, S. Wang, H. Liang, X. Hu, X. Sun, J. Zhu, L. Ma, M. Jiang, J. Hu, and J. Li (2015), A low cost surface plasmon resonance biosensor using a laser line generator. *Optics Communications* 349, 83–88.
[13] H. Li, L. Zhang, H. Cai, X. Chen, J. Sun, Y. Chao, and D. Cui (2013), Portable-surface plasmon resonance biosensor immunoassays for the human serum albumin detection. *Key Engineering Materials* 565, 408–411.
[14] H. Šípová, M. Piliarik, M. Vala, K. Chadt, P. Adam, M. Bocková, K. Hegnerová, and J. Homola (2011), Portable surface plasmon resonance biosensor for detection of nucleic acids. *Procedia Engineering* 25,148–151.
[15] B. N. Feltis, B. A. Sexton, F. L. Glenn, M. J. Best, M. Wilkins, and T. J. Davis (2008), A hand-held surface plasmon resonance biosensor for the detection of ricin and other biological agents. *Biosensors and Bioelectronic* 23, 1131–1136.
[16] K. Retra, H. Irth, and J. E. Van Muijlwijk-Koezen (2010), Surface Plasmon Resonance biosensor analysis as a useful tool in FBDD. *Drug Discovery Today: Technologies* 7,181–187.
[17] T. A. Manolio (2008), Biorepositories—at the bleeding edge. *International Journal of Epidemiology* 37, 231–233.
[18] P. Elliott, on behalf of UK Biobank, T. C. Peakman, and on behalf of UK Biobank (2008), The UK Biobank sample handling and storage protocol for the collection, processing and archiving of human blood and urine. *International Journal of Epidemiology* 37, 2, 234–244.
[19] T. C. Peakman and P. Elliott (2008), The UK Biobank sample handling and storage validation studies. *International Journal of Epidemioogy* 37, i2–i6.
[20] P. M. Baird and R. J. Frome (2005), Large-Scale Repository Design. *Cell Preservation Technology* 3, 256–266.
[21] W. Mohd, A. Wan, D. Kamarun, M. Kamil, A. Rahman, and M. S. Kamarudin (2015), Modular Surface Plasmon Resonance (SPR) Biosensor Based on Wavelength Modulation. *Advance Materials Research* 1107, 699–705.
[22] W. M. A. Wan Ahamad, D. Kamarun, and M. K. Abd Rahman (2016), Performance of newly-fabricated modular surface plasmon resonance (mSPR) sensor. *Materials Science Forum* 857, 459–464.
[23] B. Grunwald, and G. Holst (2004), Fibre optic refractive index microsensor based on white-light SPR excitation. *Sensors and Actuators A: Physical* 113, 174–180.
[24] A. Shrivastava and V. Gupta (2011), Methods for the determination of limit of detection and limit of quantitation of the analytical methods. *Chronicles Young Science* 2, 21.
[25] H. SadAbadi, S. Badilescu, M. Packirisamy, and R. Wüthrich (2013), Integration of gold nanoparticles in PDMS microfluidics for lab-on-a-chip plasmonic biosensing of growth hormones. *Biosensors and Bioelectronic* 44, 77–84.
[26] B. Liu, Y. Pang, R. Bouhenni, E. Duah, S. Paruchuri, and L. McDonald (2015), A step toward simplified detection of serum albumin on SDS-PAGE using an environment-sensitive flavone sensor. *Chemical Communications* 51, 11060–11063.