

## **Electrochemical Detection of Glycan and Protein Epitopes of Glycoproteins in Serum**

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### Published

2014

### Journal Title

Analyst

### Version

Accepted Manuscript (AM)

### DOI

[10.1039/c4an00781f](https://doi.org/10.1039/c4an00781f)

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## FULL PAPER

# Electrochemical detection of glycan and protein epitopes of glycoprotein in serum

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**ABSTRACT**

Aberrant protein glycosylation is associated with a range of pathological conditions including cancer and possess diagnostic importance. Translation of glycoprotein biomarkers will be facilitated by development of a rapid and sensitive analytical platform that simultaneously interrogates both the glycan and protein epitopes of glycoproteins in body fluids such as serum or saliva. To this end, we developed an electrochemical biosensor based on the immobilization of a lectin on the gold electrode surface to recognize/capture target glycan epitope conjugated to glycoproteins, following with detection of the protein epitope using target protein-specific antibody. Electrochemical signals are generated by label-free voltammetric or impedimetric interrogation of ferro/ferricyanide redox couple (e.g.  $[\text{Fe}(\text{CN})_6]^{3-/4-}$ ) on the sensing surface, where the change in voltammetric current or interfacial electron transfer resistance were measured. The detection system was demonstrated using the model glycoprotein chicken ovalbumin with *Sambucus nigra* agglutinin type I (SNA lectin), and exhibits femtomolar sensitivity in the background of diluted human serum. The results obtained in this proof-of-concept study demonstrate the possibility of using electrochemical detection for developing cheap point-of-care diagnostics with high specificity and sensitivity for blood glycoprotein biomarkers.

**KEYWORDS**

Electrochemical biosensor, label-free detection, Lectin, Glycoprotein biomarker, Serum, Ovalbumin

## INTRODUCTION

Differential protein glycosylation is a feature of diverse pathological conditions such as atherosclerosis, ulcerative colitis, rheumatoid arthritis, microbial infection, Alzheimer's disease and cancer.<sup>1</sup> Monitoring specific glycosylation changes for glycoprotein biomarker candidates such as fucosylated haptoglobin (pancreatic cancer) and sialylated prostate-specific antigen (prostate cancer) provides higher diagnostic power compared to changes in total glycoprotein levels.<sup>2</sup> In fact, measurement of fucosylated  $\alpha$ -fetoprotein in blood (AFP-L3 test) has been approved by the FDA for early detection of hepatocellular carcinoma.<sup>3,4</sup> Over the past few years, various glycoprotein enrichment platforms have been coupled with mass spectrometric and nuclear magnetic resonance spectroscopic techniques to uncover disease specific glycosylation changes for candidate glycoprotein biomarkers. These include single, serial and multi-lectin affinity chromatography,<sup>5,6</sup> lectin magnetic bead array (LeMBA),<sup>7,8</sup> boronic acid<sup>9</sup> and hydrazide chemistry<sup>10</sup> based extraction methods. While all these methods have excellent analytical performance in detecting candidate cancer glyco-biomarkers, they are poorly suited for routine clinical use due to high maintenance/running cost, requirement of technical expertise, long assay time and complicated data analysis procedure. The development of detection methods that are user-friendly, robust, sensitive, quick and cheaper than currently available methods is warranted to fulfill important needs for developing future diagnostics.

Electrochemical (EC) detection methods offer elegant ways for interfacing bio-recognition and transduction events and represent a substantial driver to achieve rapid, cost-effective, sensitive, selective and accurate quantification of biomolecules.<sup>11-15</sup> Among many EC methods, Faradaic electrochemical impedance spectroscopy (F-EIS) is one of the most effective methods for the label-free detection of biomolecules and for probing the build-up of the biomaterials sensing film

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3 on the electrodes.<sup>16-18</sup> In F-EIS, the binding of a target protein with its ligand (i.e., specific  
4 antibody) on electrode surface can be detected, where the change in impedance of the electrode  
5 surface and its interface to the electrolyte solution containing a redox probe (e.g.  $[\text{Fe}(\text{CN})_6]^{3-/4-}$ )  
6 is measured in the form of its electron transfer resistance ( $R_{\text{ct}}$ ).<sup>19-21</sup> This interfacial electron  
7 transfer reaction of the redox process can also be measured via voltammetric technique, where  
8 the presence or absence of the target proteins will alter the voltammetric current of the redox  
9 process at the sensing surface. More recently, the differential pulse voltammetric (DPV)  
10 interrogation of interfacial electron transfer reaction of the  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  process generated upon  
11 protein binding has been used as an effective label-free tool for protein detection.<sup>22,23</sup>

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24 In past few years, much attention has been focused on development of label-free  
25 electrochemical biosensors to monitor glycosylation changes (e.g. mannosylation,  
26 galactosylation, sialylation and fucosylation) in complex biological fluids<sup>24-27</sup> using naturally  
27 occurring lectin as glycan recognition element. These biosensors detect overall changes in glycan  
28 profile without monitoring specific glycoprotein to which glycan is attached. To increase  
29 specificity and diagnostic value, analytical methods are required to detect not only overall status  
30 of glycosylation in the sample but also aberrant glycosylation for a specific glycoprotein. To  
31 overcome this limitation, we investigated label-free electrochemical detection methods for the  
32 simultaneous interrogation of specific glycan on target glycoprotein.

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45 Here we describe the development of a simple method that can efficiently and reproducibly  
46 detect both glycan and protein epitopes of a glycoprotein in background of diluted serum sample  
47 at femtomolar concentration. The method consists of a gold macrodisk electrode that is  
48 biochemically functionalized with *Sambucus nigra* agglutinin type I lectin (SNA lectin) [specific  
49 to recognize terminal sialic acid attached to galactose through  $\alpha$ -(2-6) linkage].<sup>28</sup> The attachment  
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3 of the target glycoprotein chicken egg albumin (ovalbumin) on SNA lectin functionalized  
4 electrode and complexation with polyclonal anti-ovalbumin antibody (Figure 1 scheme) was  
5 followed by the F-EIS and DPV measurements. Each bimolecular layer on the sensor surface  
6 acts as a barrier for the interfacial electron transfer reaction of the  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  process,  
7 resulting in an increase in  $R_{\text{ct}}$  or decrease in DPV current response. The presence of target  
8 glycoprotein ovalbumin and subsequent complexation with polyclonal anti-ovalbumin antibody  
9 appear to further block the  $[\text{Fe}(\text{CN})_6]^{3-/4-}$  process from accessing the electrode surface  
10 effectively. When we monitored  $R_{\text{ct}}$  or DPV current responses generated from the  $\text{Fe}[(\text{CN})_6]^{3-/4-}$   
11 process before and after ovalbumin capture, there was a clear correlation between the presence of  
12 the target ovalbumin and changes in  $R_{\text{ct}}$  or DPV current response. To mimic clinical scenario, we  
13 spiked ovalbumin into serum and showed linear changes in current readout with concentration  
14 range from 10  $\text{pg mL}^{-1}$  to 500  $\text{pg mL}^{-1}$ . To our knowledge, this is the first electrochemical  
15 method that simultaneously interrogates specific glycans and the target protein on which the  
16 glycan is attached. Moreover, we believe the simplicity of this technology could facilitate  
17 translation of current glycoprotein biomarker research.  
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## 42 MATERIALS AND METHODS

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45 **Materials.** Biotin labeled *Sambucus nigra* agglutinin type I (SNA-I) lectin was purchased  
46 from Vector Laboratories (USA). Lyophilized chicken egg albumin (ovalbumin) (#A5503),  
47 polyclonal anti-chicken egg albumin antibody produced in rabbit (#C6534), potassium  
48 ferrocyanide, potassium ferricyanide, and KCl were purchased from Sigma (Australia).  
49 Biotinylated BSA was purchased from Thermo Scientific (USA) while multivalent streptavidin  
50 was purchased from Invitrogen (USA). Human blood sample was collected from a healthy  
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3 volunteer with consent and ethics approved by The University of Queensland Human Ethics  
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5 Committee.  
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9 **Cleaning gold disk electrodes.** Gold disk working electrodes (diameter = 3 mm) were  
10 purchased from CH Instruments (Austin, USA). The electrodes were reused after cleaning and  
11 regeneration. The electrodes were cleaned using piranha ( $\text{H}_2\text{SO}_4:\text{H}_2\text{O}_2 = 3:1$ ) on a sonicator  
12 water-bath for 30 sec - 1 min (Note: piranha is a highly toxic and hazardous chemical. It is to be  
13 handled in fume-hood with adequate personal protective equipments. University safety  
14 guidelines were followed for its disposal). The electrodes were then physically polished with 1  
15 micron alumina and subsequently with 0.05 micron alumina slurry. Prior to electrochemical  
16 cleaning, electrodes were sonicated in acetone for 20 min. Electrochemical cleaning was  
17 performed in 0.5 M  $\text{H}_2\text{SO}_4$  until reproducible characteristic gold electrode profiles were  
18 achieved.  
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33 **Construction of biosensor.** Immediately before functionalization, thoroughly cleaned gold  
34 disk electrodes were dried under a flow of nitrogen gas. The electrodes were incubated with 500  
35  $\mu\text{g mL}^{-1}$  biotin-BSA in 1 $\times$  PBS (137 mM NaCl, 2 mM KCl, 10 mM phosphate buffer, pH 7.4) on  
36 thermo shaker set at 25 C, 300 rpm for 45 min. The electrodes were washed with 1 $\times$  PBS after  
37 each incubation step for 3 times. The electrodes were then incubated with multivalent  
38 streptavidin (500  $\mu\text{g mL}^{-1}$  in 1 $\times$  PBS, at 25 C, 300 rpm for 45 min), followed by biotinylated  
39 SNA lectin (500  $\mu\text{g mL}^{-1}$  in 150 mM NaCl containing 0.1 mM  $\text{Ca}^{2+}$ , at 25 C, 300 rpm for 45  
40 min) to form the bio-recognition layer of lectin on electrode surface. Ovalbumin stock solution  
41 was made at concentration of 2 mg  $\text{mL}^{-1}$  and stored at -30 C in aliquots. Designated  
42 concentration of ovalbumin was freshly made (in 150 mM NaCl solution containing 0.1 mM  
43  $\text{Ca}^{2+}$ ) prior to use and protein capture was performed for 45 min with gentle agitation using  
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3 intelli-mixer (PCOD Scientific). 1 in 1000 dilution of polyclonal anti-ovalbumin antibody in 1×  
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5 PBS for 15 min was used for detection of captured chicken egg albumin. Designated  
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7 concentrations of ovalbumin were spiked in diluted serum (1 in 1000 dilution of serum in 150  
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9 mM NaCl solution containing 0.1 mM Ca<sup>2+</sup>).  
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13 After modification of the sensor surfaces (up to SNA lectin attachment), the actual detection  
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15 steps include capture of the ovalbumin antigen followed by the anti-ovalbumin detection. Both of  
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17 these steps are directly followed by the label-free impedance (or DPV) technique. Thus once  
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19 electrode is modified with SNA lectin, overall detection process takes approximately 2 hr.  
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22 **Electrochemical measurement.** All electrochemical experiments were conducted at room  
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24 temperature (25 ± 1°C) in a standard three-electrode electrochemical cell arrangement using an  
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26 electrochemical workstation CHI 650D, CH Instruments (Austin, USA). The electrochemical  
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28 cell consisted of gold disk electrode sensor as a working electrode, platinum wire counter  
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30 electrode and Ag/AgCl (3M KCl) reference electrode. Electrochemical signals were measured in  
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32 1× PBS buffer (pH 7.4) containing 2.5 mM [Fe(CN)<sub>6</sub>]<sup>3-</sup>/ [Fe(CN)<sub>6</sub>]<sup>4-</sup> (1:1) and 0.1 M KCl. DPV  
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34 signals were obtained with a potential step of 4 mV, pulse amplitude of 50 mV, pulse width of  
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36 200 ms, sampling width of 16.7 ms and pulse period of 500 ms. The F-EIS spectra were  
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38 recorded in 1× PBS buffer (pH 7.4) containing 2.5 mM [Fe(CN)<sub>6</sub>]<sup>3-</sup>/ [Fe(CN)<sub>6</sub>]<sup>4-</sup> (1:1) and 0.1 M  
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40 KCl using an alternating current voltage of 5 mV, with the frequency range of 0.1 (or 1) Hz –  
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42 100 kHz. During run, bias DC current was applied below frequency of 100 Hz. The faradic  
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44 current generated by the K<sub>3</sub>[Fe(CN)<sub>6</sub>]/K<sub>4</sub>[Fe(CN)<sub>6</sub>] probe accounts for the presence of a  
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46 biomolecule. The current changes corresponding to ovalbumin detection were calculated as  
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48 follows:  
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$$\% \text{ Decrease of DPV peak current} = (I_{\text{before}} - I_{\text{after}}) \times 100 / I_{\text{before}} \dots\dots (1)$$

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6 where  $I_{\text{before}}$  = peak current at ovalbumin capture step [*e.g.*, current recorded at biotin-  
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8 BSA/multivalent streptavidin/biotinylated SNA lectin/designated concentration of ovalbumin  
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10 (either in buffer or spiked into 1 in 1000 serum)],  $I_{\text{after}}$  = peak current at detection step [*e.g.*,  
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12 current recorded at biotin-BSA/multivalent streptavidin/biotinylated SNA lectin/designated  
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14 concentration of ovalbumin (either in buffer or spiked into 1 in 1000 serum)/1 in 1000 anti-  
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16 ovalbumin polyclonal antibody].  
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20 For each electrode, peak current was normalized with DPV response obtained at initial step when  
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22 electrodes were clean.  
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## 28 **RESULTS & DISCUSSION**

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31 **Construction of biosensor.** Figure 1 describes assembly scheme of the biosensor. As reported  
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33 previously,<sup>18</sup> biotin labeled BSA is used for coating gold electrode surface. Subsequently,  
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35 multivalent streptavidin was used as a linker to immobilize biotin labeled SNA lectin to form  
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37 bio-recognition layer on the electrode surface. The formation of bio-recognition layer on  
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39 electrode surface affects analytical performance of the biosensor and in our experiments this  
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41 formation is controlled by attachment of biotin-BSA, streptavidin and biotinylated SNA lectin.  
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43 To achieve maximal analytical performance, we optimized binding conditions for all three  
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45 biomolecules one after another by incubating electrodes with three different concentrations of  
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47 biotin-BSA, multivalent streptavidin and biotinylated SNA lectin.  
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53 Firstly to determine optimal biotin-BSA concentration, we incubated thoroughly cleaned gold  
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55 electrodes with  $100 \mu\text{g mL}^{-1}$ ,  $500 \mu\text{g mL}^{-1}$  and  $1000 \mu\text{g mL}^{-1}$  solution of biotin-BSA for 45 min.  
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57 The electrodes were washed three times with  $1\times$  PBS, followed by F-EIS measurements to  
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3 determine optimal biotin-BSA concentration. As compared to  $100 \mu\text{g mL}^{-1}$ , incubation of  
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5 electrodes with  $500 \mu\text{g mL}^{-1}$  concentration of biotin-BSA showed increase in size of the  
6  
7 semicircle on the Nyquist plot (Figure 2A, i vs. ii) suggesting increased electron transfer  
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9 resistance ( $R_{ct}$ ). There was no further increase in  $R_{ct}$  at  $1000 \mu\text{g mL}^{-1}$  biotin-BSA concentration  
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11 as compared to  $500 \mu\text{g mL}^{-1}$  (Figure 2A, ii vs. iii). As  $500 \mu\text{g mL}^{-1}$  biotin-BSA showed best F-  
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13 EIS i.e. maximum  $R_{ct}$  response, the same binding condition was used for subsequent  
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15 experiments. Next, F-EIS for three different concentrations  $100 \mu\text{g mL}^{-1}$ ,  $500 \mu\text{g mL}^{-1}$  and  $1000$   
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17  $\mu\text{g mL}^{-1}$  of multivalent streptavidin under optimal biotin-BSA binding conditions was obtained.  
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19 As shown in Figure 2C, incubation of electrode with  $500 \mu\text{g mL}^{-1}$  concentration of multivalent  
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21 streptavidin showed maximum  $R_{ct}$  under optimal biotin-BSA binding condition. Similarly, as  
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23 shown in Figure 2E,  $500 \mu\text{g mL}^{-1}$  of biotinylated SNA lectin showed best F-EIS response under  
24  
25 optimal biotin-BSA and multivalent streptavidin binding conditions. To confirm F-EIS results  
26  
27 we conducted parallel DPV measurements and as shown in Figure 2B, 2D and 2F, there was  
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29 decrease in peak DPV current corresponding with increase in  $R_{ct}$  suggesting reduction in actual  
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31 electron transfer between electrode/redox electrolyte double layer with increased resistance. For  
32  
33 all three biomolecules, maximum  $R_{ct}$  and minimal DPV peak current was observed at  $500 \mu\text{g}$   
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35  $\text{mL}^{-1}$  concentration. Moreover, there was no further increase in  $R_{ct}$  at higher concentration i.e.  
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37  $1000 \mu\text{g mL}^{-1}$ . This suggests that electrode surface is getting saturated when incubated with  $500$   
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39  $\mu\text{g mL}^{-1}$  concentration of biomolecules and there is no non-specific binding of the protein  
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41 directly on the electrode surface at higher concentration. As the protein binding to electrode  
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43 surface is showing saturation without any non-specific binding, it is anticipated that there will be  
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45 minimal or no non-specific binding of biomolecule of interest directly to the electrode surface  
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47 during further stages of experiment.  
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3 **Glycoprotein capture and detection.** After optimizing conditions for SNA lectin  
4 immobilization on electrode surface, next we captured glycoprotein ovalbumin in 150 mM NaCl  
5 solution containing 0.1 mM  $\text{Ca}^{2+}$ . The formation of bio-recognition layers and capture of  
6 ovalbumin was followed by electrochemical detection. As shown in Figure 3A, biotin-BSA  
7 coated electrode gave rise to smallest semicircle (Figure 3Ai), followed by streptavidin (Figure  
8 3Aii), SNA lectin (Figure 3Aiii) and ovalbumin (Figure 3Aiv) showed gradual increase in  
9 semicircle indicating increase in electron transfer resistance. In line with changes observed in  
10 impedance, we observed corresponding decrease in peak DPV current (Figure 3B). These results  
11 firstly confirm results shown in previous section for successful formation of bio-recognition  
12 layers on electrode surface. Next, it demonstrates successful capture of target glycoprotein  
13 ovalbumin on SNA biosensor with electrochemical detection.  
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29 Lectins generally recognize glycan structures with low affinity but with high avidity mainly  
30 through hydrogen bonding, hydrophobic interactions and van der Waals forces.<sup>29</sup> In fact, glycan  
31 structure specific interactions of a glycoprotein with lectins are very well demonstrated in the  
32 recent past mainly using sialic acid binding lectin biosensors.<sup>25-27, 30</sup> Incubation of SNA lectin  
33 biosensor with asialofetuin, a non-sialic acid expressing glycoform variant of glycoprotein  
34 fetuin, showed very minimal change in the baseline impedance.<sup>25-27, 30</sup> On contrary, a linear  
35 increase from baseline impedance was observed when SNA lectin biosensor was incubated with  
36 increasing concentration of fetuin expressing correct glycan epitope.<sup>25-27, 30</sup> Apart from showing  
37 very high binding specificity towards glycan epitope, these SNA lectin biosensors have  
38 demonstrated up to attomolar sensitivity for the glycoprotein detection (e.g. fetuin).<sup>25-27, 30</sup> SNA  
39 along with other electrochemical lectin biosensor developed so far have been applied to glycan  
40 profiling of healthy and rheumatoid arthritis human serum samples,<sup>27</sup> monitor glycosylation  
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3 changes between normal and cancerous pancreatic cell extracts,<sup>25</sup> dengue diagnosis<sup>31</sup> and  
4 microorganism recognition,<sup>32</sup> without detecting specific glycoproteins. Detection of both glycan  
5 and protein epitopes of a glycoprotein is required to provide the specificity to measure  
6 glycosylation-specific biomarker, for example fucosylated alpha-fetoprotein. Therefore,  
7 following capture of ovalbumin glycoprotein using SNA lectin biosensor, we interrogated  
8 ovalbumin protein backbone using polyclonal anti-ovalbumin antibody. Upon capturing  
9 ovalbumin on SNA biosensor, electrodes were incubated with 1 in 1000 dilution of anti-  
10 ovalbumin antibody, followed by F-EIS and DPV measurements. As compared to ovalbumin  
11 capture step (Figure 3Aiv) incubation of electrode with anti-ovalbumin antibody (Figure 3Av)  
12 showed increase in impedance, with corresponding decrease in DPV peak current (Figure 3B).  
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14 These results indicate stepwise construction of bio-recognition layer, capture of ovalbumin on  
15 SNA biosensor and detection of ovalbumin using polyclonal anti-ovalbumin antibody. As F-EIS  
16 and DPV techniques showed an excellent agreement between the results obtained for stepwise  
17 binding of biomolecules on the sensor surface, we presented only DPV responses for remaining  
18 all experiments.  
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38 As antibodies are glycoprotein themselves, they may directly interact with lectin which may  
39 lead to false positive results. Therefore, contribution of false positive response due to direct  
40 interaction between SNA lectin and polyclonal anti-ovalbumin antibody was tested. The gold  
41 electrode modified with biotin-BSA/streptavidin/SNA lectin was directly incubated with anti-  
42 ovalbumin antibody to allow any possible direct interaction between them. As shown in Figure  
43 3C we did not observe any change in DPV response between biotin-BSA/streptavidin/SNA lectin  
44 and biotin-BSA/streptavidin/SNA lectin incubated with anti-ovalbumin antibody. This finding  
45 indicates that there was no direct interaction between ovalbumin antibody with SNA lectin.  
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3 Thus, we demonstrate stepwise formation of bio-recognition layer, capture of glycoprotein  
4 ovalbumin on SNA biosensor surface followed by detection of ovalbumin using anti-ovalbumin  
5 antibody and label-free electrochemical detection using F-EIS and DPV measurements.  
6  
7 Moreover, we did not observe any direct interaction between lectin and antibody which excludes  
8 possibility for false positive results. To the best of our knowledge, this is the first  
9  
10 electrochemical biosensor which interrogates both glycan epitope of a glycoprotein using lectin  
11 and protein epitope using anti-protein antibody with label-free electrochemical monitoring.  
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20 **Detection of ovalbumin in serum.** To evaluate the biosensor performance under more  
21 physiological conditions, we conducted a series of experiments in which ovalbumin was spiked  
22 into diluted human serum collected from healthy donor. To mimic biomarker detection  
23 condition, ovalbumin at final concentration of  $1 \text{ ng mL}^{-1}$  was spiked into 1:1000 serum sample.  
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25 Similar dilution of serum sample has been recommended to study overall changes in glycan  
26 profiles using electrochemical detection.<sup>27</sup> Blood is very heterogeneous in terms of its protein  
27 composition, consisting of proteins with abundance varying several orders of magnitude. Top six  
28 abundant plasma proteins account for 85% of the total plasma proteome.<sup>33</sup> Out of them, five are  
29 found to be glycosylated (transferrin, haptoglobin,  $\alpha$ 1-antitrypsin, IgG, and IgA) and show  
30 varying degree of binding to SNA lectin. This means that one would expect significant amount  
31 of glycoprotein capture when electrode immobilized with SNA lectin is incubated with  
32 serum/plasma sample. As can be seen in Figure 4A, DPV peak current was significantly reduced  
33 when SNA lectin modified electrode was incubated with serum sample (Figure 4A, i vs. ii).  
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35 However, there was no further decrease in DPV response with anti-ovalbumin antibody  
36 incubation suggesting the antibody did not bind non-specifically with any SNA lectin-bound  
37 human serum proteins (Figure 4A, ii vs. iii).  
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3 When ovalbumin was spiked into serum, incubation of electrode with polyclonal anti-  
4 ovalbumin antibody (Figure 4Bii) showed significant reduction in peak DPV response as  
5 compared to DPV peak current resulted at the ovalbumin capture step in serum (Figure 4Ai).  
6  
7 This data clearly demonstrates that our method is highly effective for specific detection of  
8 ovalbumin glycoprotein in the presence of large excess of several other SNA lectin binding  
9 proteins in serum.  
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13 To assess the dynamic range and lower limit of detection of our method, a dilution series of  
14 ovalbumin protein ( $1 \text{ pg mL}^{-1}$  to  $10 \text{ ng mL}^{-1}$ ) was spiked in diluted serum. For each ovalbumin  
15 concentration, electrochemical response was measured at the step of glycoprotein capture and  
16 detection step using anti-ovalbumin antibody. The percentage change in peak current between  
17 these two measurements is attributed to the amount of ovalbumin present in the sample. As  
18 shown in Figure 4C, there was a gradual increase in percentage change in DPV peak current with  
19 increasing concentration of ovalbumin spiked in serum ranging from  $10 \text{ pg mL}^{-1}$  to  $500 \text{ pg mL}^{-1}$ .  
20 Beyond  $500 \text{ pg mL}^{-1}$ , there was no further increase in DPV response suggesting saturation of the  
21 glycoprotein capture on electrode surface. The lower limit of detection of  $10 \text{ pg mL}^{-1}$  described  
22 here is comparable with previous electrochemical glycan profiling studies.<sup>25, 27</sup> This  $10 \text{ pg mL}^{-1}$   
23 lower limit of detection in 1000 fold diluted serum sample will actually translate into  $10 \text{ ng mL}^{-1}$   
24 in the actual undiluted sample. Previously published lectin electrochemical biosensors showed  
25 femtomolar sensitivity and outperformed conventional lectin-ELISA and lectin-microarray by  
26 several fold in terms of lower limit of detection.<sup>25, 27</sup> Unlike previous studies which only  
27 interrogated glycan, here we interrogated both glycan as well as protein epitope of a glycoprotein  
28 in background of diluted serum with detection limit of  $10 \text{ pg mL}^{-1}$ . Detection limit of our assay  
29 could be further improved via incorporating and optimizing the device geometry and  
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3 experimental parameters.<sup>34</sup> Moreover, designing multiplexed electrochemical biosensor<sup>25, 34</sup>  
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5 would help to monitor panel of clinically relevant glycoprotein biomarkers in parallel.  
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## 10 CONCLUSION

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14 In this proof-of-concept study we demonstrated interrogation of glycan along with protein  
15 epitope of a glycoprotein ovalbumin using SNA lectin and anti-ovalbumin antibody respectively  
16 followed by label-free electrochemical detection in background of diluted serum. Our lectin-  
17 antibody biosensor detects specific glycan and the glycoprotein to which the glycan is attached,  
18 hence providing increased specificity over existing lectin biosensors. Furthermore, with detection  
19 limit of 10 pg mL<sup>-1</sup>, lectin-antibody electrochemical biosensor described here could be developed  
20 further to achieve point-of-care diagnosis for clinically relevant glycoprotein biomarkers.  
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## 30 Notes

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32 The authors declare no competing financial interest.  
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## 36 Acknowledgments

37  
38  
39 AKS is supported by International Postgraduate Research Scholarship (IPRS) and UQ  
40 Centennial (UQ Cent) Scholarship. This work was supported by the ARC DECRA  
41 (DE120102503) to MJAS. We also acknowledge funding received by our laboratories from the  
42 National Breast Cancer Foundation of Australia (CG-12-07) to MT, NC-14-022 to MH and MT.  
43  
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48 These grants have significantly contributed to the environment to stimulate the research  
49 described here.  
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**FIGURE CAPTIONS**

**Figure 1. (A)** Schematic illustration of the preparation of an SNA lectin immunosensing layer. **(B)** Schematic view of the glycoprotein ovalbumin capture and label-free detection using anti-ovalbumin antibody which results in increased charge transfer resistance ( $R_{ct}$ ) and corresponding decrease in DPV peak current.

**Figure 2.** Optimization of biosensor construction. Nyquist plots of a gold electrode modified with (A) biotin-BSA, (C) biotin-BSA/streptavidin, and (E) biotin-BSA/streptavidin/SNA lectin in  $1\times$  PBS buffer containing 2.5 mM  $K_3[Fe(CN)_6]$ , 2.5 mM  $K_4[Fe(CN)_6].3H_2O$  and 0.1 M KCl. (B, D, F) Corresponding DPV responses obtained at a gold electrode modified with (B) biotin-BSA, (D) biotin-BSA/streptavidin, and (F) biotin-BSA/streptavidin/SNA lectin, respectively. The concentration of biotin-BSA in Figures A & B, multivalent streptavidin in Figure C & D (following incubation with  $500\ \mu g\ mL^{-1}$  of biotin-BSA), and SNA lectin in Figure E & F (following incubation with  $500\ \mu g\ mL^{-1}$  biotin-BSA and  $500\ \mu g\ mL^{-1}$  streptavidin each) were (i)  $100\ \mu g\ mL^{-1}$ , (ii)  $500\ \mu g\ mL^{-1}$ , and (iii)  $1000\ \mu g\ mL^{-1}$ .

**Figure 3. (A)** The Nyquist plots and **(B)** Differential pulse voltammetric response for monitoring (i) Biotin-BSA (ii) Biotin-BSA/Multivalent Streptavidin (iii) Biotin-BSA/Multivalent Streptavidin/SNA lectin (iv) Biotin-BSA/Multivalent Streptavidin/SNA lectin/ $1\ ng\ mL^{-1}$  ovalbumin and (v) Biotin-BSA/Multivalent Streptavidin/SNA lectin/ $1\ ng\ mL^{-1}$  ovalbumin/anti-ovalbumin antibody in  $1\times$  PBS buffer containing 2.5 mM  $K_3[Fe(CN)_6]$ , 2.5 mM  $K_4[Fe(CN)_6].3H_2O$  and 0.1 M KCl. **(C)** Differential pulse voltammetric response to detect any non-specific binding of anti-ovalbumin antibody with SNA lectin (i) Biotin-BSA/Multivalent

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3 Streptavidin/SNA lectin and (ii) Biotin-BSA/Multivalent Streptavidin/SNA lectin/anti-  
4 ovalbumin antibody.  
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9 **Figure 4.** Differential pulse voltammetric response for **(A)** Detection of SNA binding proteins  
10 present in human serum (i) Biotin-BSA/Multivalent Streptavidin/SNA lectin (ii) capturing SNA  
11 lectin binding glycoproteins present in human serum (1 in 1000 dilution) and (iii) detection of  
12 any non-specific recognition of SNA bound serum glycoprotein using anti-ovalbumin antibody.  
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16 **(B)** Detection of ovalbumin spiked in human serum (i) capturing  $1 \text{ ng mL}^{-1}$  ovalbumin in human  
17 serum (1 in 1000 dilution) using SNA lectin and (ii) its detection using anti-ovalbumin antibody.  
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21 **(C)** Calibration plot for % change in differential pulse voltammetric response for detection of  
22 designated concentration of ovalbumin spiked in human serum (1 in 1000 dilution) using anti-  
23 ovalbumin antibody. Each data point represents average  $\pm$  SD (standard deviation) of 3  
24 independent experiments.  
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FIGURES

Figure 1

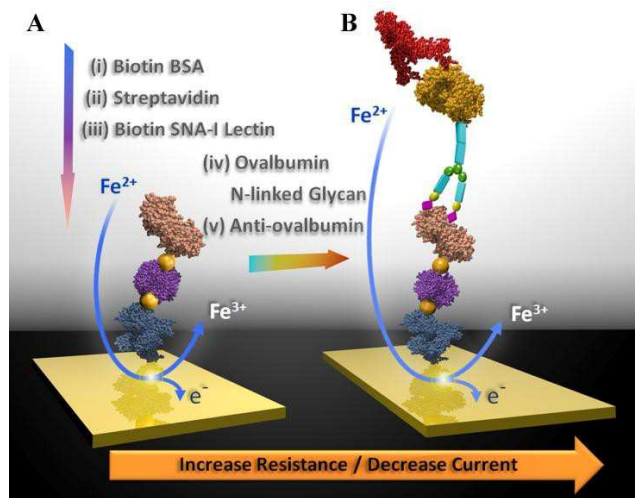


Figure 2

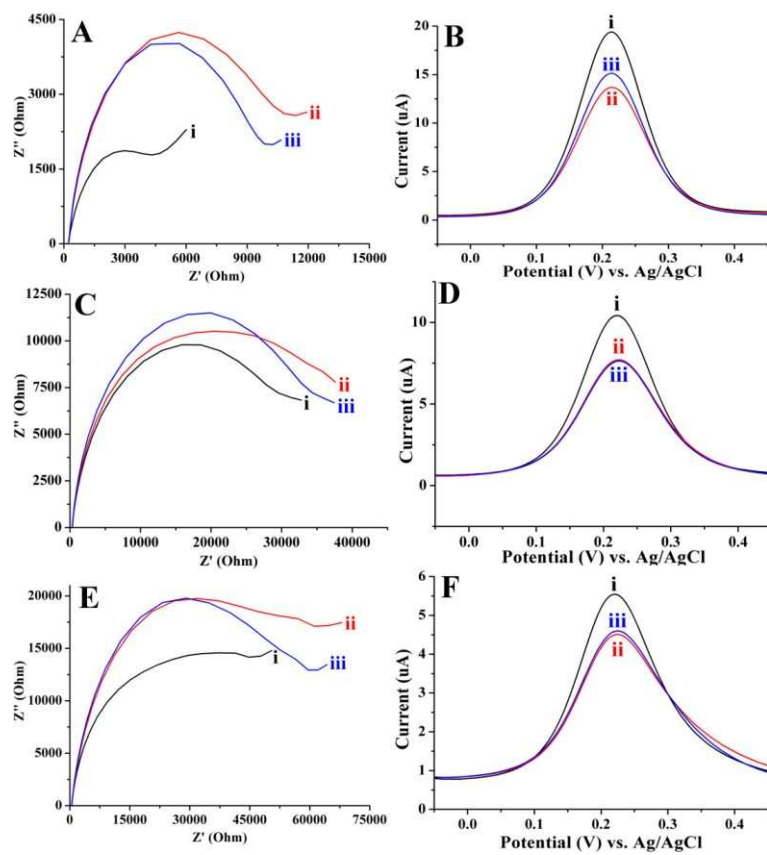


Figure 3

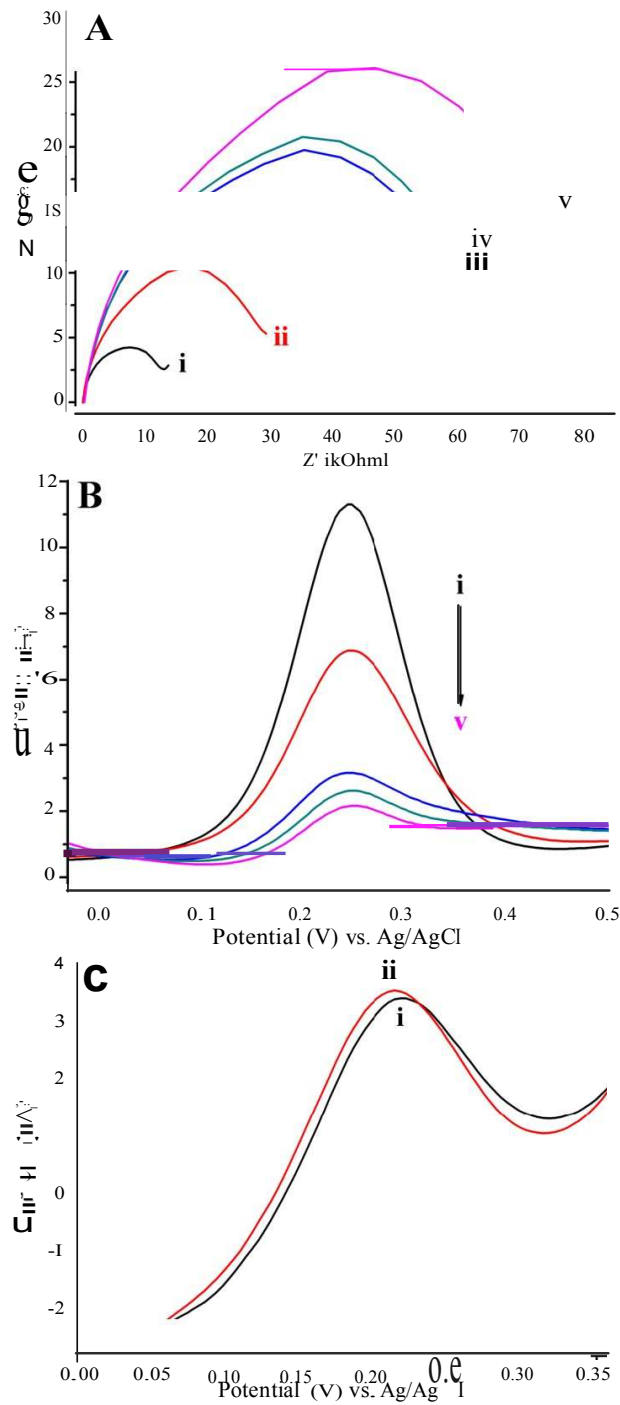
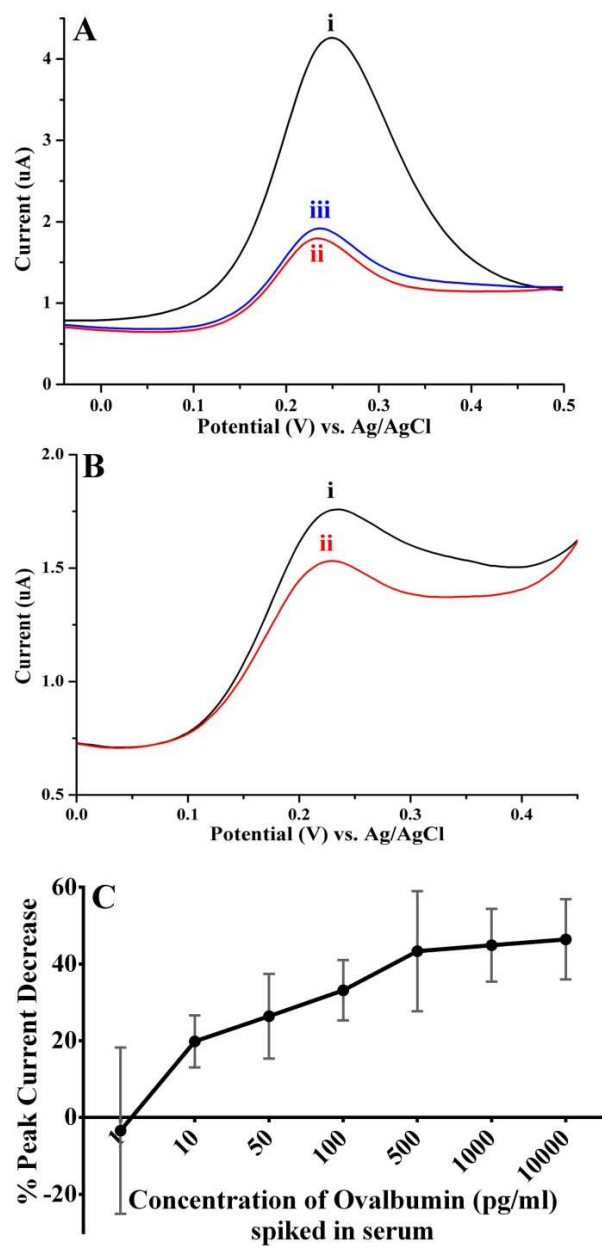
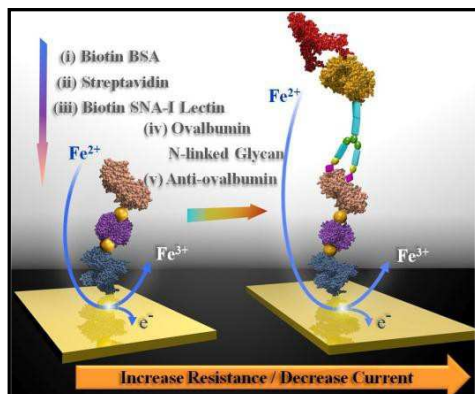


Figure 4



## Table of contents entry



**Description:** Electrochemical interrogation of glycan and protein epitopes of glycoprotein chicken ovalbumin using SNA lectin and anti-ovalbumin antibody in serum



# Analyst

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