Supplementary Information

Direct Correlation between Potentiometric and Impedance Biosensing of Antibody-Antigen Interactions using an Integrated System

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Electrical measurements

The *I-V* characteristics of the EGFET biosensors were measured with a Keithley 4200-SCS semiconductor characterization system. The V_d was 0.2 V and a positive V_{ref} sweep was applied to the liquid through a commercial dip-in Ag/AgCl reference electrode (Princeton Applied Research / Ametek #K0260). The impedance spectra of EIS biosensors were measured with a Gamry Interface 1000 potentiostat. The DC bias was -0.4 V and the RMS AC oscillation was 5 mV. Cyclic voltammetry was used for the deposition of the o-ABA functional layer on the sensing surface. The voltage scan range is 0 to 0.8 V for 10 scans and the scan rate was 50 mV/s.

Analysis of EIS biosensors for BSA/anti-BSA system

The plot of R_{ct} and $C_{surface}$ values versus anti-BSA concentration are shown in Figure S1a and S1b, respectively. R_{ct} responds well to the increasing concentration of anti-BSA. However, the surface capacitance shows a much smaller response to various anti-BSA concentrations after a large drop of capacitance value upon the introduction of 10 ng/ml anti-BSA solution.

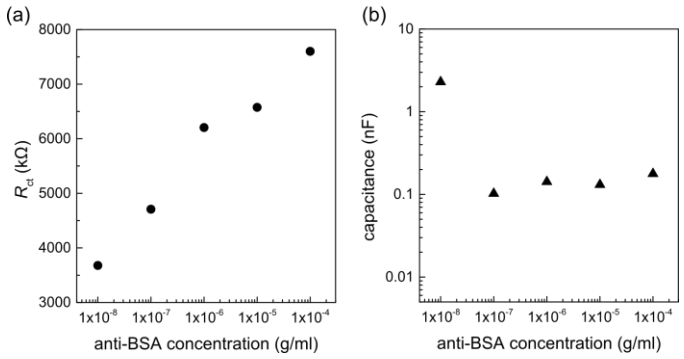

Figure S1. The values of circuit elements from the fitting of measured impedance spectra versus different anti-BSA concentrations. (a) The plot of R_{ct} versus different anti-BSA concentrations. (b) The plot of $C_{surface}$ versus different anti-BSA concentrations.

Detection of bovine parainfluenza using HN/BPIV3 blood system

Beyond the prototype BSA/anti-BSA system in simple buffer solution, animal disease diagnosis using serological samples from animal blood was also demonstrated. Bovine parainfluenza virus type-3 (BPIV3), a common cause of pneumonia in cattle, was detected in blood plasma samples by immobilization of hemagglutinin-neuraminidase (HN) as the probe on the sensing surface. HN was produced using the baculovirus expression system using previously established protocols.[1] The WE was coated with o-aminobenzoic acid (o-ABA, 50 mM in H_2SO_4) carboxylated film through 10 cyclic voltammetry scans (0 to 0.8 V, 50 mV/s). Similar to the above-mentioned BSA immobilization, HN proteins (50 μg/ml in acetate buffer pH 4.0) were immobilized to the sensing surface through amine coupling. Blood samples taken from calves in Northern Ireland were processed to plasma[2] and screened for the presence of anti-BPI3V antibodies using the Svanovir PI3V-Ab ELISA. The sensing surface was blocked using 1M ethanolamine followed by diluted bovine plasma (50X dilution in HBS-EP buffer, pH 7.4) after the immobilization of HN proteins. The HN-immobilized sensing surface was then exposed to dilutions of positive blood plasma in HBS-EP buffer for 30 min contact time. After the contact, the sensing surface was rinsed with HBS-EP buffer to remove the weakly bound antibodies and possible non-specific binding. The electrical measurement was performed in buffer with 10 mM hexaammineruthenium (III) chloride in HBS-EP as the redox couple. Figure S2a shows the Nyquist plot of the EIS impedance biosensors in response to several dilutions of positive BPIV3 plasma samples. When the charged BPIV3 HN attaches to the sensing surface, the biomolecular attachments cause the change of surface potential measured by the FET as well as a change of the impedance measured by EIS biosensors. Figure S2b shows a linear relationship between the exponentiation of surface potential change (measured by FET) and the charge transfer resistance (from EIS). Again, the linear relationship fits well into the Butler-Volmer theory, confirming the correlation between potentiometric and impedance biosensors and that the surface potential change is the origin of sensing response for both sensing techniques.

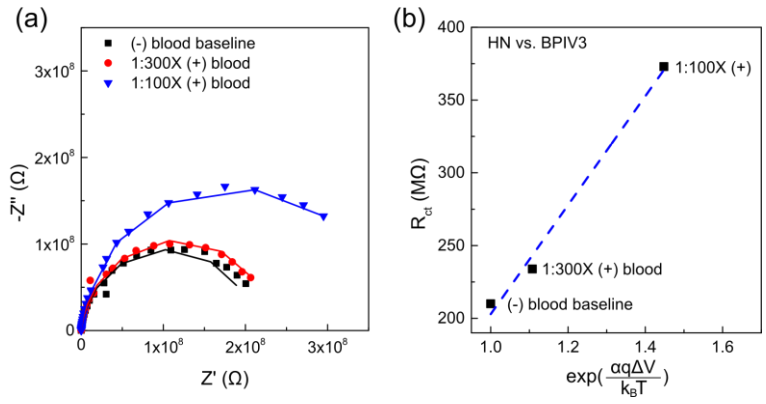

Figure S2. The sensing response of HN vs. BPIV3 using integrated biosensor system (a) The Nyquist plot of impedance spectra after the sensing surface exposed to different BPIV3 antibody dilutions. (b) The relationship between exponentiation of ΔV and the R_{ct}. A linear relationship is observed and agrees with the prediction of Butler-Volmer theory.

References

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