Supplementary Materials for

Design of molecular imprinting biosensor with multi-scale roughness for bio-molecular detection

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Electrochemical impedance spectroscopy (EIS) characterization

Electrochemical impedance spectroscopy (EIS) measurements were performed using a CHI 660d electrochemical workstation (CH Instruments) with a three electrodes system. It consists of a gold chip electrode, with or without molecular imprinting, as the working electrode, a platinum wire as the counter electrode and an Ag/AgCl electrode (BASi) as the reference electrode.

In Figure 1(F), thiol titration experiment was utilized to characterize surface coverage by thiol molecules after adding thiol solution into detection solution. Obviously, larger OCP response can be observed on rough gold surface compared to smooth gold surface, indicating the much larger gold surface area existed on the rough gold surface. This can be further proven by EIS. In Figure S1, bare gold substrate displayed a straight line, corresponding to a mass diffusion-limited electron transfer process. After the co-deposition of thiols, the straight line was converted to a well-defined semicircle. Dimension of this semicircle corresponds to the value of impedance. Clearly, the impedance on rough gold SAM is much larger than on smooth gold SAM, which means more SAM generated on rough gold surface than smooth gold surface.

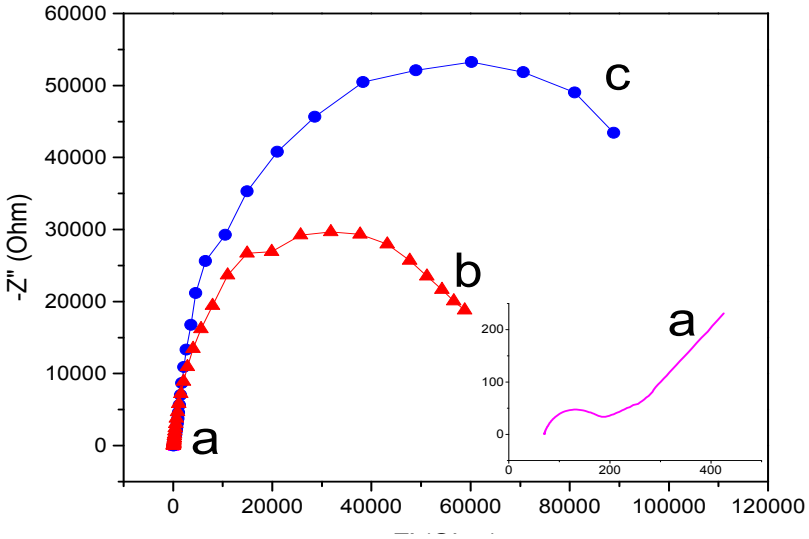

Fig. S1 Electrochemical impedance spectra (EIS) of different electrodes. (a) bare gold, (b) SAM coated after thiol titration on b) smooth gold surface and c) rough gold surface. EIS was tested with 5 mV amplitude and frequency range of 0.1-100,000 Hz. EIS was performed in KCl solution (0.1M) containing $Fe(C1N)_6^{3-/4-}$ (5 mM).

Detection of myoglobin

Hb and Mb are the smallest analytes of the current set which were tested. Hence they are also ideal for exploring the sensitivity of biosensor to surface roughness. The OCP response of electrodes imprinted on the smooth gold surface for Mb and probed with Mb and Hb are shown in Fig S2. No response is obtained when the analyte is chemically different from the one with which the electrode was imprinted. The maximum OCP response is also compared in the bar graph, which shows no response can be observed when Mb is imprinted on rough surface. However, when Mb is imprinted on smooth gold surface, not only large OCP response can be obtained in the detection process, but also the specificity can be well proved as well. The performance of Mb biosensor was greatly improved in the smooth surface. On the surface feature, both horizontal distance and vertical distance, are much larger than the size of hemoglobin molecule, which is only 3.5 ×2 × 4.5 nm. Only the bottom side of the Mb molecule can be imprinted with the SAM. However, on the smooth gold surface, the horizontal and vertical feature is much smaller than the rough gold surface, which can greatly help the Mb molecule fit into the crevice on the smooth gold surface. Much more surface fraction is imprinted with SAM, and leaves enough thiol pattern on the surface after washing step, which greatly improve the recognition ability during the detection step. Hence, the smooth gold surface is great for small molecule, which fits most of the protein.

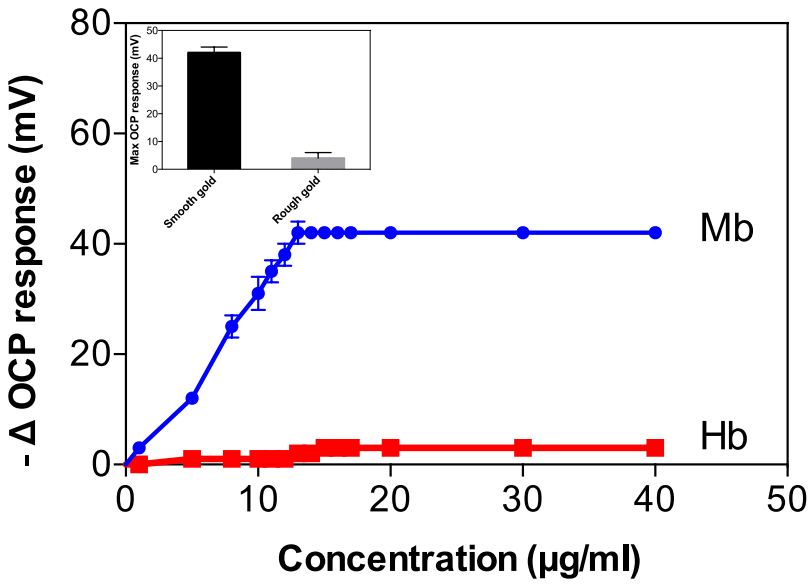

Fig. S2 Mb biosensor cross test with Mb and Hb on smooth gold surface. Inset: comparison of max OCP response of Mb biosensor on smooth gold surface and rough gold surface.

Detection of CEA

CEA and MMP7 are well-established markers for breast, colon, and pancreatic cancers. Detection of CEA and MMP 7 can help doctors make rapid point of care, clinical decisions for patient. Currently, the most common detection method is of CEA detection is ELISA, which is time consuming, expensive and complicated.

In Figure S3, the maximum OCP of CEA imprinted on smooth and rough gold is compared, showing that the optimal OCP response is achieved only when imprinting is done on the smooth gold surface. Since CEA is a cylindrical molecule, 8 nm x 27 nm in size, we can clearly see that it is a very good match, both in the vertical and horizontal directions of the smooth surface as opposed to the rough surface, where it is dwarfed in both horizontal and vertical directions. In the inset shows we show the cross test result between the two molecules. On the smooth surface, the CEA imprinted biosensor generated no OCP response with the MMP 7 analyte, and conversely no response was observed with the CEA analyte when the sensor was imprinted for MMP7.

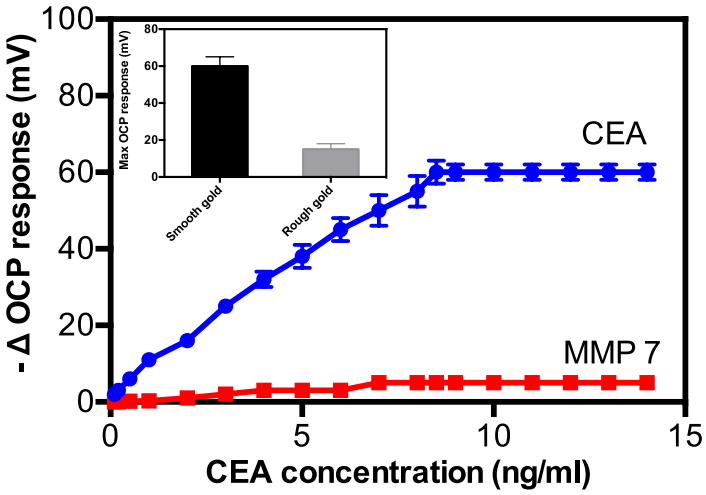

Fig. S3 CEA biosensor cross test with CEA and MMP 7 on smooth gold surface. Inset: comparison of max OCP response of CEA biosensor on smooth gold surface and rough gold surface.

Hb real time OCP detection

Potentiometer was employed to monitor the OCP response for the analyte detection. The example of how the final analyte concentration vs - Δ OCP response was present in Fig. S4. It is clear that the -Δ OCP response increases with the addition of Hb detection solution. As the surface cavities were fully occupied by Hb analyte, the OCP response reaches the maximum value. Hb concentration in detection beaker vs - Δ OCP response was converted from the original Time vs – OCP response data. In the inset of Fig. S4, a completed OCP response process was monitored by potentiometer. Hb detection solution was added into detection beaker stepwise by micropipette, and the OCP response was recorded by potentiometer. The corresponding Hb concentration for each addition was labeled by red arrow in the figure. Through the data monitored by potentiometer, the Hb detection process can be easily recorded and analyzed.

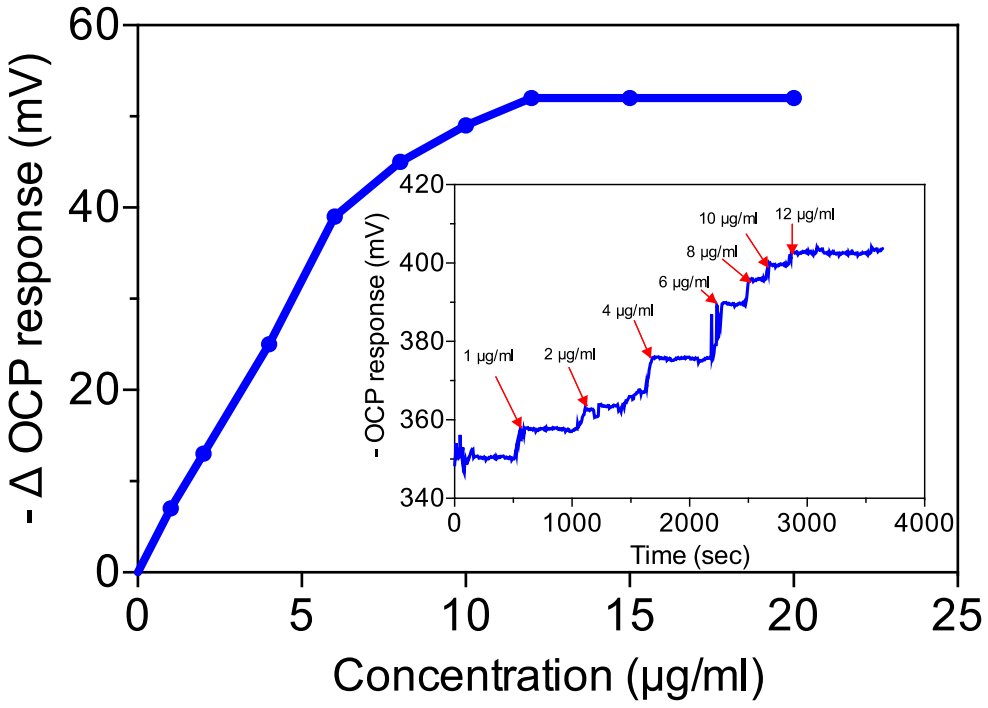

Figure S4. Hb real time OCP detection. OCP response as a function of Hb concentration added into testing beaker. Inset was the original Time-OCP response data. The OCP response was plotted as a function of time period. The dynamic process of OCP detection can be monitored through potentiometer.