Preparation of Electrochemical Aptamer-based Sensors: A direct Aryl Diazonium Grafting Approach

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RESULTS SECTIONS

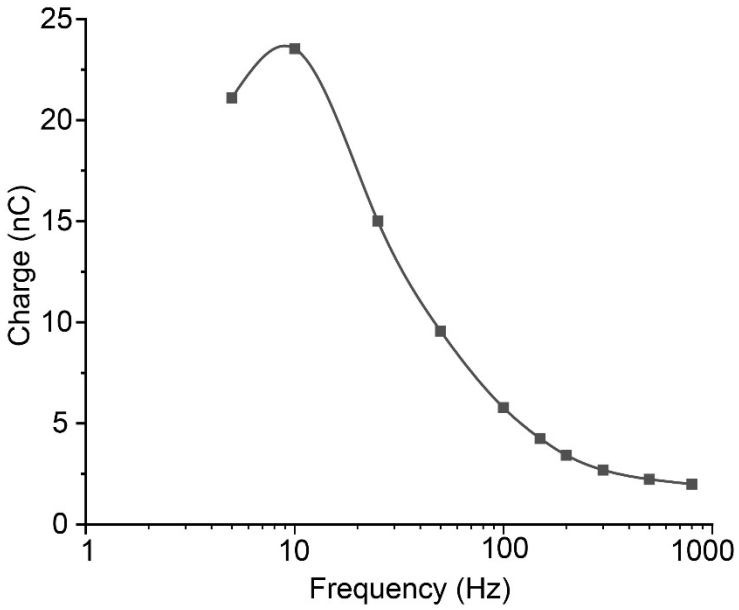

Figure S1. Charge-frequency plot for the thiol-gold enabled aptamer sensor.

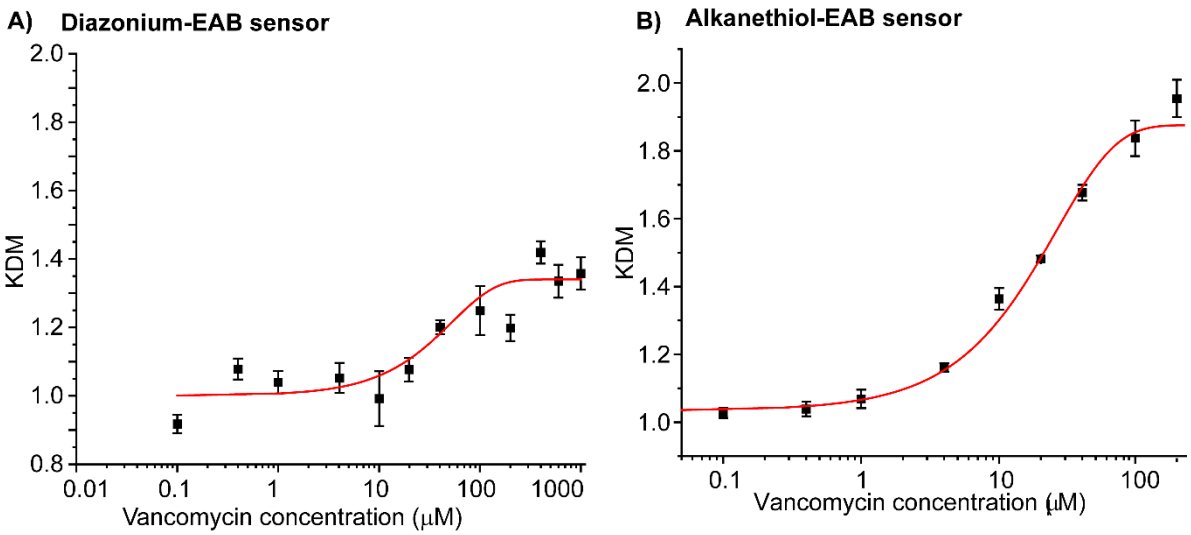

Figure S2. Calibration curves for vancomycin EAB sensors made using the diazonium chemistry approach (A) and the conventional alkanethiol approach (B) showing the sensor obtained using the aryldiazonium approach follows a logarithmic signal increase pattern.

EXPERIMENTAL SECTION

Chemical reagents and Materials

All chemicals were purchased of high-purity grades. Sodium hydroxide (NaOH) ≥98%, pellets (anhydrous), sulfuric acid 98% and sodium nitrite 99.999% and hydrochloric acid (HCl) ACS reagent, 37% and magnesium chloride hexahydrate were supplied from Sigma-Aldrich. Milli-Q® was used to prepare all the aqueous solutions. Gold wires (0.25 mm diameter), >99.999%, were purchased from Goodfellow (UK). Leakless Ag/AgCl (3 M KCl) reference electrodes and platinum counter electrodes were purchased from CH Instruments. Phosphate buffered saline (PBS) was obtained from ThermoFisher Scientific (Australia) and used after adding 2 mM $MgCl_2$ to mimic the concentration of magnesium in blood. An oligonucleotide with a 4-aminobenzoic acid (4-ABA) functional group at the 5' end and a methylene blue moiety at the 3' end was acquired from Eurogentec (Liège, Belgium). The DNA sequence in this work (Table S1) was designed to target the antibiotic vancomycin. Miniature heat-shrink tubing was bought from McMaster-Carr, Illinois, USA.

Table S1. Sequence and functional groups of the aptamer used.

−4-ABA−O−PO_2O-GCGAGGGTACCGCTTAAAGTGGGTCGGC−O-CH_2−CH(CH_2 OH) −NH−C(O)−$(CH_2)_2$−MB−3′

Gold electrode cleaning/treatment before functionalisation

The Au wires were cut into 6 cm long electrodes that were then covered with a 4.5 cm long miniature heat shrink tubing with 0.5 cm of the Au wire is kept bare at one end and used as the working electrode area. The gold wire electrode working area was electrochemically cleaned by successive cycling voltammetry scanning from −1 to −1.6 V in 0.5 M NaOH for 200 cycles at a scan rate of 1 V/S. The NaOH scanning was then followed by another cleaning step by voltammetry cycling in 0.5 M sulfuric acid for 30 cycles between −0.2 V and +1.6 V at a scan rate of 0.1 V/S. The gold wires were then washed with Milli-Q water before immersion in the DNA containing solution.

Gold electrode functionalisation via in situ diazotisation of arylamine-terminated aptamers.

The aptamer grafting solution was prepared from a 0.7 mM $MgCl_2$/1xPBS buffer. The gold wire electrodes were immersed in 1 μM solution of 4-aminobenoic acid terminated aptamer for 1 hour at °C (note: the 4-ABA group is located at the 5' end and connected to the aptamer nucleobases via an amide bond, while the methylene blue moiety is located at the 3' end of the aptamer). After the aptamer attachment step, the gold electrodes were immersed in 5 mM solution of 6-mercapto-1-hexanol (MCH) in 0.7 $MgCl_2$/1xPBS buffer for 3 hours. For the alkanethiol terminated aptamer EAB sensors, the clean Au wires were incubated in the S-S-terminated aptamer solution, that was submitted to TCEP (1 mM) for 1 h in dark, for 1 hour, before immersing the electrode in 5 mM MCH for 3 hours.

Electrochemical measurements and data analysis

All fabricated sensors were electrochemically interrogated using square wave voltammetry (SWV) using Multichannel Autolab potentiostat (Metrohm). Electrochemical measurements were carried out using a three-electrode electrochemical cell system with Ag/AgCl as the reference electrode, Pt wire as the counter electrode and the DNA functionalised gold wire as the working electrode.[1] Data analysis was performed using in home-built Matlab code that fitted the square wave voltammetry peaks, extracted the absolute peak currents and charges, normalised current values and calculated KDMs values. SWV was measured from −0.45 V to −0.05 (versus Ag/AgCl) with an amplitude of 25 mV in PBS buffer containing 0.7 mM $MgCl_2$ (pH = 7.4) at 28 °C. The sensors were left in the measurement buffer (at 28 ˚C) for 5 minutes before SWV recoding to allow for the temperature to equilibrate. Different concentrations of vancomycin were spiked in the measurement buffer followed by 3-5 minutes of stirring before SWV recording.

The working area of the gold electrode was controlled via limiting the physical working area of the gold wire to 0.5 cm long and the effective working area of these electrodes was controlled by applying a two-step electrochemical cleaning process by first scanning the electrodes in NaOH (0.5 M) for 200 cycles followed by

cleaning in sulfuric acid (0.5 M) for 30 cycles. Integrating the reduction peaks in the cyclic voltammograms of the bare gold electrodes in H_2SO_4 shows that the variation in the effective surface area of the different gold electrodes used in the experiment is insignificant. The effective working area of the sensing layer was measured by integrating the reduction peak of the electrochemical cleaning cyclic voltammetry signal of the gold wire in sulfuric acid (0.5 M) as shown in Figure 1b. We have allowed the formation of a single monolayer of oxygen on the bare gold electrode by voltametric scanning from −0.1 to +1.6 V (vs. Ag/AgCl) in 0.5 M sulfuric acid, followed by reducing that monolayer by scanning back from +1.6 V to −0.1 V (Figure S3A). We then integrated the area under the reduction peak to calculate the charge (Figure S3) and that charge was divided by 400 mC cm^{-2}, which corresponds to the charge density of an oxygen monolayer on gold (equation 1).

$$A_{eff} = Q/q_{red} \qquad \text{equation S1}$$

Where A_{eff} = Effective surface area (cm^2) of the gold electrode, Q = Charge from the gold oxide reduction peak (Coulombs) calculated by integrating the reduction peak in cyclic voltammogram of the gold electrode cleaning in H_2SO_4 (Figure S3A), and q_{red} = Theoretical Charge density for the gold oxide reduction (≈400 µC/cm^2 or 0.0004 C/cm^2). Figure S3C shows calculated effective surface area of five different gold electrodes with the effective surface area variation within ±10% suggesting with minimum variations from electrode to electrode. Error bars represent the standard deviations of the area calculated from reduction peak last five cyclic voltammograms of the H_2SO_4 electrochemical cleaning

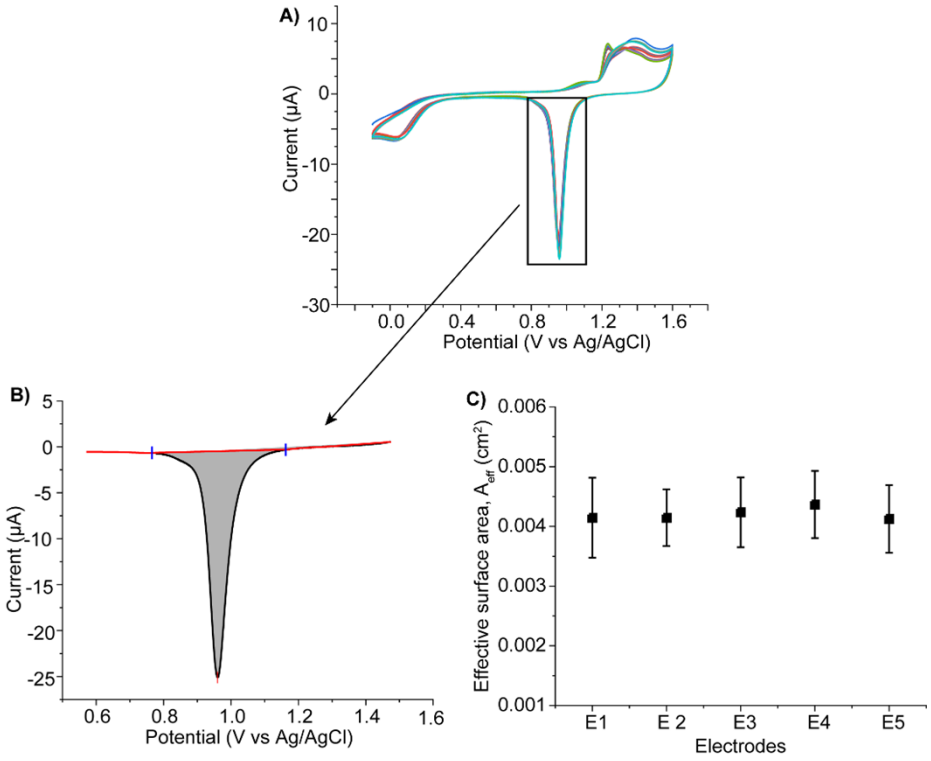

Figure S3. Cyclic voltammetry of different four gold electrodes in that were cleaned in H_2SO_4 (0.5 M) for 30 cycles between −0.1 V and +1.6 V (vs Ag/AgCl). Note, here we put only the last 5 cycles of every cleaning to avoid data overlay. Data shows that the variation of reduction peak area/current is minimum between the different gold electrodes. B) Reduction peak integration that was used to calculate the charge and thus the effective surface area of the different gold electrodes. C) Charge of the different clean gold electrodes obtained by integrating the reduction peaks of the H_2SO_4 cleaning, showing the variations between the different electrode's charges/effective area is minimum (~10%) between the different electrodes. Note, error bars in Figure S2C represent the standard deviation of the last five cyclic voltammograms of the cleaning process.

For SWV data analysis, the peak currents were extracted for the different target concentrations at the different interrogation frequencies. The voltametric peak currents were then converted to normalised signal change by calculating the difference between the current at certain vancomycin concentration and the current in the absence of vancomycin (Equation S1).

$$Normalised\ signal = \frac{I\ (target) - I\ (blank)}{I\ (blank)} \qquad \text{(equation S2)}$$

Then KDM was calculated by measuring the normalised signal difference at two frequencies (One high frequency where the sensor has a signal-on and one low frequency where the sensor has a signal-off) for each target concentration, see equation S2.

$$KDM\ (\%) = \frac{Normalised\ signal\ at\ high\ ftrequency - Normalised\ signal\ at\ low\ frequency}{1 + \left(\frac{Normalised\ signal\ at\ high\ ftrequency - Normalised\ signal\ at\ low\ frequency}{2}\right)} \times 100$$

(equation S3)

References

1. R. Lao, S. Song, H. Wu, L. Wang, Z. Zhang, L. He and C. Fan, *Anal. Chem.*, 2005, **77**, 6475-6480.