Electronic Supplementary Material (ESI) for Analyst.

Switching of electrochemical characteristics of redox protein upon specific biomolecular interactions

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Supporting Information

Materials and Methods

1 Sample preparation

Cleaning of bulk gold electrode

Gold disk working electrodes with radius 1mm (CH Instrument, Austin, TX, USA) were sonicated for 5 minutes in 3% decon, rinsed with deionised water (DI), followed by electrochemical reductive desorption in 0.5M NaOH from -0.9 to -1.9 V versus Hg/Hg_2SO_4 until no further changes in the voltammogram were observed. The electrodes were then polished for 5 minutes with 0.3µm aluminium oxide powder (Buehler, Lake Bluff, IL, USA) on a polishing pad (Buehler). The residual powders were removed by sonicating in DI water for 5 minutes, polishing on a blank polishing pad, and finally sonicating again in DI water for another 5 minutes. Electrochemical cleaning in 0.5M H_2SO_4 was performed by scanning the potential between -0.05V and +1.1V versus Hg/Hg_2SO_4 for 55 cycles.

Electrode funtionalization

Electrodes were incubated with 20µl of 20µg/ml biotinylated Az for 2 hours, followed by backfilling with 10µl of 100µM thiolated polyethylene glycol (PEG) S(CH2)11(OCH2CH2)3OH.

Specific interaction and control

For specific interaction, the functionalized electrodes were incubated with 20µl of 20µM streptavidin in 10mM pH7 phosphate buffer for 1 hour. As a control, electrodes were incubated with 10µg/ml Ovalbumin specific scFv antibody in PBS for 1 hour (provided by DSTL).

2 AC voltammetry measurement

Measurements were performed in a three-electrode cell, with Hg/Hg_2SO_4 (K_2SO_4 sat.) reference electrode and Pt counter electrode, using an Autolab PGSTAT302 potentiostat. The potential scan ranged from -0.45 V to 0.45V vs Hg/Hg_2SO_4. The amplitude and frequency of the superimposed rms signal were 10mV and 2Hz, respectively.

3 Electrochemical Impedance Spectroscopy (EIS) measurement

Measurements were performed in a three-electrode cell, with Hg/Hg_2SO_4 (K_2SO_4 sat.) reference electrode and Pt counter electrode, using an Autolab PGSTAT302 potentiostat. The measuring buffer contains 1mM $K_4[Fe(CN)_6]$ + 1mM $K_3[Fe(CN)_6]$ in phosphate buffer pH 7 for a range of ionic strength specified in the experiments. The impedance spectrum was measured from 0.1Hz to 10KHz, with 10mV ac potential superimposed on a dc bias potential equals to the formal potential of the redox couple (-0.21V vs Hg/Hg_2SO_4). The charge transfer resistance, R_{ct}, was determined by fitting the data to a Randles equivalent circuit.

4 Quartz crystal microbalance (QCM) measurement

The substrate of QCM was a gold-coated (mirror finish) 9MHz AT-cut quartz crystals with 2.5mm radius. In first approximation, neglecting any viscosity changes, the resonant frequency of the crystal changes with the mass loading according to the Sauerbrey equation:

$$\Delta\text{mass} = \frac{-\Delta f \times A \times \sqrt{\mu_q \times \rho_q}}{2 \times F_q^2}$$

where Δmass : Mass change
Δf : Resonant frequency change
μ_q : AT-cut quartz crystal constant (2.947×10^{11} g cm^{-1} sec^{-2})
ρ_q : Quartz crystal density (2.648 g cm^{-3})
F_q : Reference frequency (9.00 MHz)
A : Quartz crystal surface area (0.196 cm^2)

QCM measurements were performed with QCA922 instrument from Princeton Applied Research. A 2.3ml Teflon cell was used. A magnetic stirrer was positioned in the cell to ensure proper mixing of the injected targets. However this mechanical vibration contributed noise to the measurement. A gold-coated crystal was held vertically in the cell to prevent non-specific deposition of biomolecules by gravitational forces. The quartz crystal was cleaned using acetone vapour and UV/ozone.

Immobilization of biotinylated Azurin

Figure 1 shows the real-time QCM measurement during biotinylated Azurin immobilization. A frequency change of 22Hz was observed, which corresponded to a change of mass of 23.5ng. The molecular weight of biotinylated Az is estimated to be 15.8kDa. yielding a coverage of 4.6 x 10^{12} cm^{-2}.

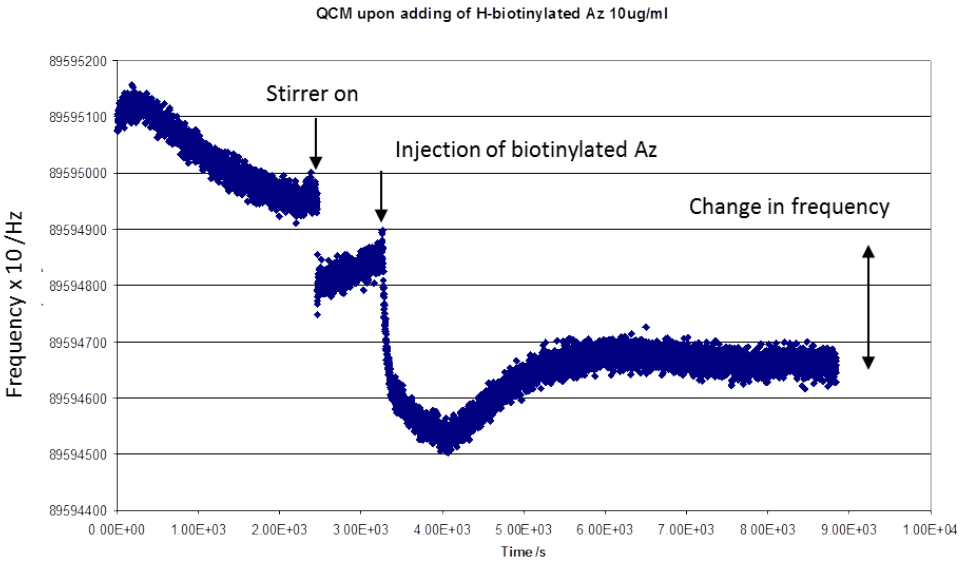

Figure 1 QCM for immobilization of biotinylated Azurin

Reaction with Streptavidin

Figure 2 shows the QCM measurement for the reaction between biotinylated Az and Streptavidin. A frequency change of 9Hz was observed, which corresponded to a change of mass of 9.61ng. The molecular weight of biotinylated Az is estimated to be 60kDa. yielding a coverage of 4.9 x 10^{11} cm^{-2}. The theoretical maximum coverage of streptavidin, given its size (5.5nm in diameter), is 4.2 x 10^{12} cm.

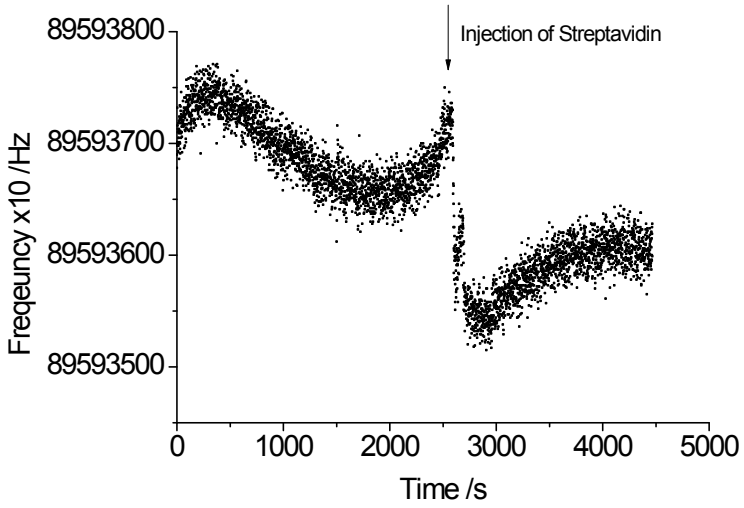

Figure 2 QCM for reaction of biotinylated Az with Streptavidin

5 Fitting parameters for several electrodes under different conditions:

i) Biotinylated Azurin
ii) Biotinylated Azurin after incubation with control
iii) Biotinylated Azurin after incubation with streptavidin ($\sigma > -(\sigma_M + \sigma_{PET})$)

Content	Electrode	$(\sigma_M + \sigma_{PET})$/ C m^{-2}	σ/ C m^{-2}
Biotinylated Az	1	0.0269	-0.0308
	2	0.0269	-0.0296
	3	0.0268	-0.0294
	4	0.0274	-0.0330
Antibody	5	0.0276	-0.0287
	6	0.0269	-0.0312
Streptavidin	7	0.0271	-0.0252
	8	0.0260	-0.0218

Table 1 Fitting parameters for electrodes under different conditions