Electronic Supplementary Information (ESI)

Online regeneration of piezoelectric and impedimetric immunosensor for the detection of C-reactive protein onto oriented antibody gold surface

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1 Materials and reagents

Monoclonal mouse anti-human CRP (MCA5880G, mAb capture antibody), polyclonal goat anti-human CRP (1707-0189G, pAb detection antibody), native human CRP (1707-2029) were purchased from AbD Serotech. 4-Mercaptophenylboronic acid (MPBA), D-glucose (non-specific blocking reagent), absolute ethanol (EtOH), potassium ferrocyanide $K_4Fe(CN)_6$, Potassium ferricyanide $K_3Fe(CN)_6$, were purchased from Sigma Aldrich. Sulphuric acid, potassium chloride, hydrogen peroxide (30%) were purchased from ACE chemicals (South Africa). Phosphate buffer saline (PBS, 0.010 M, pH 7.4) containing monobasic potassium phosphate, sodium chloride, and dibasic potassium phosphate was used. Ultrapure water with a resistivity of 18.2 MΩ.cm obtained from Milli-Q water purification system (Millipore Corp, Bedford, USA) was used throughout the experiment.

2 Equipment

All electrochemical measurements were carried out using the AUTOLAB PGSTAT 302N Electrochemical Workstation interfaced to a proline desktop computer equipped with a 1.10 version NOVA. A three-electrode system was used for all the electrochemical measurements. The electrochemical data was collected using a gold disk electrode as working electrodes (r = 0.8 mm) purchased from BASi (USA), platinum wire as counter electrode and silver|silver chloride Ag|AgCl 3M KCl solution as reference electrode. Electrochemical Impedance Spectroscopy (EIS) experiments were recorded in the frequency range between 10 kHz to 100 mHz at the half-wave potential ($E_{1/2}$) of (1:1) 2.0 mM $K_3/K_4[Fe(CN)_6]$ redox couple for bare gold with an amplitude 5 mV rms sinusoidal modulation.

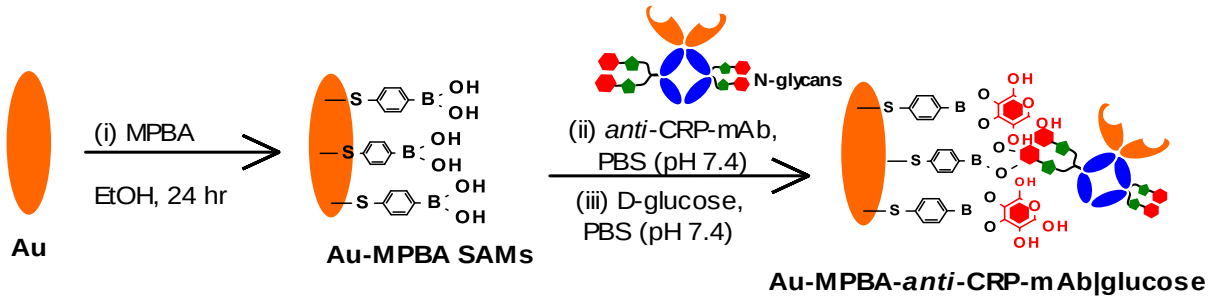
3 Cleaning of AuCQC sensor chips and MPBA SAMs modification

Prior to SAM modification, the AuCQC sensor chips were cleaned by immersing the crystals in a solution of NH_3:H_2O_2:H_2O in a ratio of 1:1:5 and heated at 75°C for 10 minutes. The pre-treated crystals were rinsed thoroughly with copious amounts of ultrapure Milli-Q water. Afterwards, the crystals were cleaned by exposing to ozone-producing ultraviolet light (UV-O) for 5 minutes providing the final removal of organic contaminants before the crystals could be used for QCM-D measurement. Quartz crystals were modified *ex-situ* with 4-mercaptophenylboronic acid as stated in Section 2.3. The AuCQC-MPBA SAM modified crystals were mounted in titanium chambers and used with an E4 QCM-D sensor system (Q-Sense, Sweden). The gold coated crystals QSX-301 (Au-CQC, 5 MHz AT-cut and a diameter of 14 mm were used) was purchased from QSense, (Sweden). The chamber temperature was set at 25°C. QCM-D analysis made use of an Ismatec peristaltic pump set

on a flow rate of 50 μL.min^{-1}. The estimated mass was calculated using Sauerbrey and Kelvin-Voigt models to estimate the mass of the adsorbed molecule. The Voigt model was fitted using the using the QTools software.

4 Immobilization procedure/sensor fabrication, Scheme S1

Gold disc electrode (Au, BASInc) was used for the electrochemical impedance spectroscopy (EIS) immunosensor fabrication and gold-coated quartz-crystal (AuCQC, Q-sense®, Sweden) was used for quartz-crystal microbalance with dissipation (QCM-D) immunosensor fabrication. The gold surfaces (Au or AuCQC) were immersed in 5 ml absolute ethanol solution containing 4-mercaptophenylboronic acid (5.0 mM) for 24 hr at room temperature to form MPBA self-assembled monolayer (Au/AuCQC-MPBA SAM). The Au/AuCQC-MPBA SAM formation for the QCM-D was accomplished *ex-situ* due to the incompatibility of the QCM-D tubing to organic solvents (absolute ethanol). The immobilization of the capture *anti*-CRP monoclonal antibody (*anti*-CRP-mAb, 5.0 μg.mL^{-1} in 0.010 M PBS, pH 7.4) was achieved via the boronate ester reaction with the N-glycans on the Fc region of the *anti*-CRP-mAb. The unreacted boronic acid sites were blocked by reacting with D-glucose solution (30 μg.mL^{-1} in 0.010 M PBS, pH 7.4) to give Au/AuCQC-MPBA-*anti*-CRP-mAb|glucose. **Scheme S1** shows the representation of the fabrication of immunosensors for gold (Au) surface (gold disc electrode or gold-coated quartz crystal) Au/AuCQC-MPBA-*anti*-CRP-mAb|glucose.



Scheme S1 Fabrication of immunosensors using gold (Au) surface (gold disc electrode for impedimetric) and gold-coated quartz crystal (AuCQC) for mass-sensitive (QCM-D) signal generation, represented respectively as Au/AuCQC-MPBA-*anti*-CRP-mAb|glucose.

The design of the CRP sensing gold surface was modified following the step-by-step procedure shown in **Scheme S1** and as described in the experimental section above. The gold disk electrode (BASi) with 0.80 mm radius was used. The method for the immobilization of anti-CRP-mAb afforded the oriented immobilization via site-specific glycosylation reaction. The unreacted 1,2-diol reactive surfaces from the Au-MPBA SAM was blocked with glucose. The step-by-step modification of gold surfaces was followed cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The inhibition of the electron transfer properties of the bare gold electrode surface upon modification in the presence of the redox probe was used as a measure of surface functionalization.

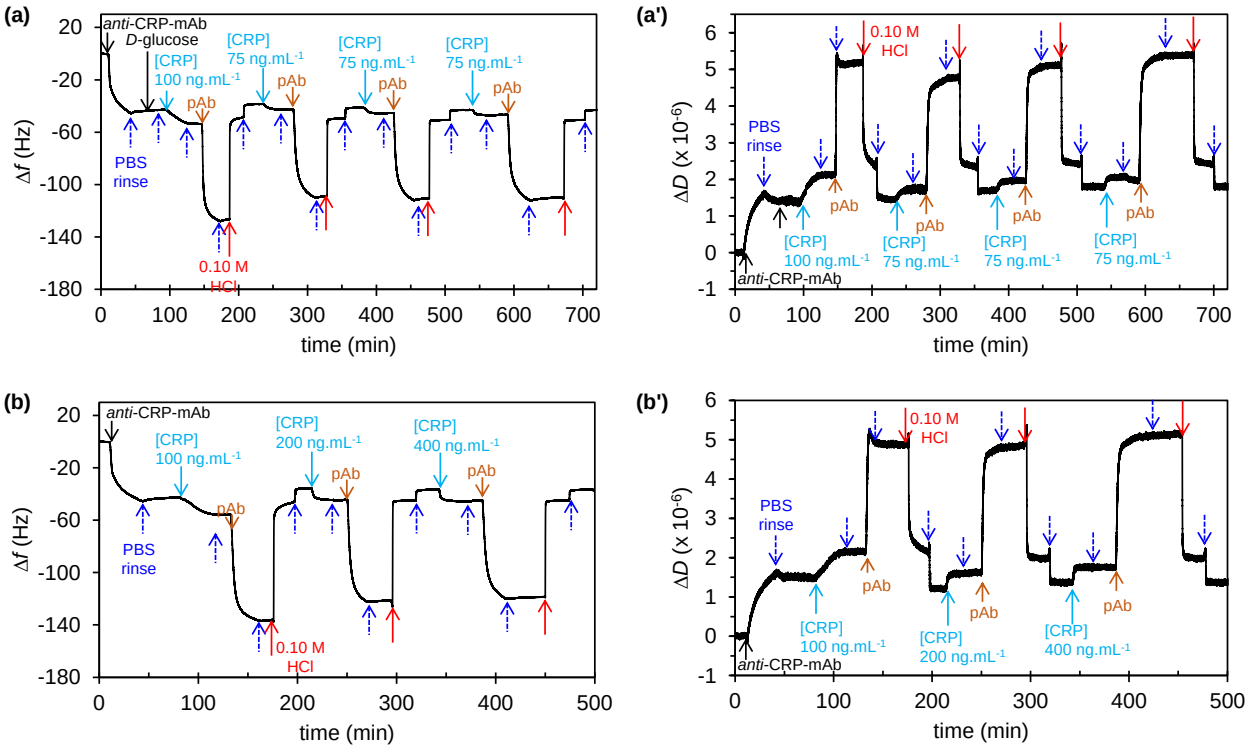


Fig. S1 Sensorgrams for different CRP concentrations: (a) 75 ng.mL^{-1} and 100 ng.mL^{-1} and (b) 100 ng.mL^{-1}, 200 ng.mL^{-1}, and 400 ng.mL^{-1}, Δf (Hz) and corresponding ΔD. The capture of CRP was followed by the capture of pAb (*anti*-CRP-pAb). After each sensing cycle, the rinsing with 0.010 M PBS (pH 7.4) and surface regeneration using 0.10 M HCl were conducted.

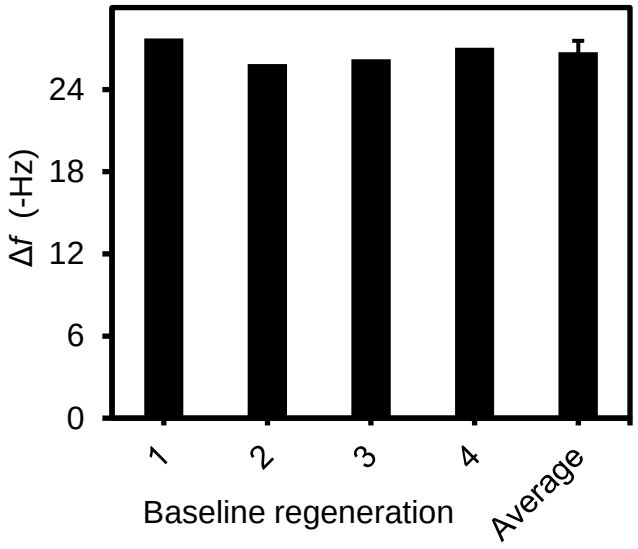


Fig. S2 Baseline regeneration of the Au-MPBA-*anti*-CRP-mAb|glucose immunosensor.

Table S1: Kelvin-Voigt and Sauerbrey parameters obtained from Q-tools modelling software for different [CRP] using Au-MPBA-*anti*-CRP-mAb|glucose. [*anti*-CRP-pAb] = 30 μg.mL^{-1}. (n = 3)

	Kelvin-Voigt			**Sauerbrey**	
Immobilized layer	Viscosity (kg.ms^{-1})	Mass/area (ng.cm^{-2})	Thickness (nm)	Mass/area (ng.cm^{-2})	Thickness (nm)
CRP (10 ng.mL^{-1}) Δf_7= -1.54 Hz ΔD_7= 0.61x10^{-7}	0.000059	40	0.11	27.25	0.07
***anti*-CRP-pAb** **Δf_7= -30 Hz** **ΔD_7 = 1.85x10^{-6}**	0.00062	740	7.50	525.00	5.60
CRP (25 ng.mL^{-1}) Δf_7= -2.9 Hz ΔD_7= 0.24x10^{-6}	0.000079	80	0.20	51.33	0.12
***anti*-CRP-pAb** **Δf_7= -37.4 Hz** **ΔD_7 = 1.88x10^{-6}**	0.00091	940	8.00	662.00	6.93
CRP (50 ng.mL^{-1}) Δf_9= -5.24 Hz ΔD_9= 0.33x10^{-6}	0.000086	110	1.10	92.75	0.30
***anti*-CRP-pAb** **Δf_9= -53 Hz** **ΔD_9 = 2.61x10^{-6}**	0.00103	1080	10.00	938.10	7.92
CRP (75 ng.mL^{-1}) Δf_9= -7.7 Hz ΔD_9= 0.4x10^{-6}	0.00017	255	1.24	136.30	0.79

***anti*-CRP-pAb** **Δf_9= -68 Hz** **ΔD_9 = 3.11x10^{-6}**	0.0015	1370	13.58	1203.60	11.98
CRP (100 ng.mL^{-1}) Δf_9= -10.2 Hz ΔD_9= 0.62x10^{-6}	0.00029	350	4.30	180.54	1.96
***anti*-CRP-pAb** **Δf_9= -78.2 Hz** **ΔD_9 = 3.25x10^{-6}**	0.0025	1510	15.10	1384.14	14.36
CRP (200 ng.mL^{-1}) Δf_7= -8.8 Hz ΔD_7= 0.48x10^{-6}	0.00018	300	3.00	155.80	1.62
***anti*-CRP-pAb** **Δf_7= -74.2 Hz** **ΔD_7 = 3.33x10^{-6}**	0.0020	1470	14.80	1313.34	13.62
CRP (400 ng.mL^{-1}) Δf_7= -8.40 Hz ΔD_7= 0.46x10^{-6}	0.00013	270	2.60	148.68	1.34
***anti*-CRP-pAb** **Δf_7= -70.1 Hz** **ΔD_7 = 3.31x10^{-6}**	0.0017	1440	14.50	1240.77	13.21

5 Stability and reproducibility of AuCQC-MPBA-*anti*-CRP-mAb|Glucose

The fabricated immunosensor was very stable due to the covalent attachment of *anti*-CRP-mAb onto boronate ester surface. The immobilization of the *anti*-CRP-mAb onto the sensor surface was tested on the quartz crystal with the RSD of 1.96% thereby confirming the stability. The reproducibility of the immunosensor was tested using four different

concentrations of the CRP-antigen as shown in **Fig. S3**. The concentrations are (10, 50, 75, and 100 ng.mL^{-1}) of the CRP-antigen. The relative standard deviations (RSD) were calculated both for the direct CRP in **Fig. S3(a)** and enhancement of CRP using *anti*-CRP-pAb at varying [CRP] in **Fig. S3(b)**. The good RSD values ranging from 0.54 to 5.54 for direct CRP and 2.08 to 7.59 for enhancement of CRP using *anti*-CRP-pAb were an indication of the good reproducibility of the fabricated immunosensors. The response of the developed immunosensor was reproducible and stable. The RSD values are presented in **Table S2**.

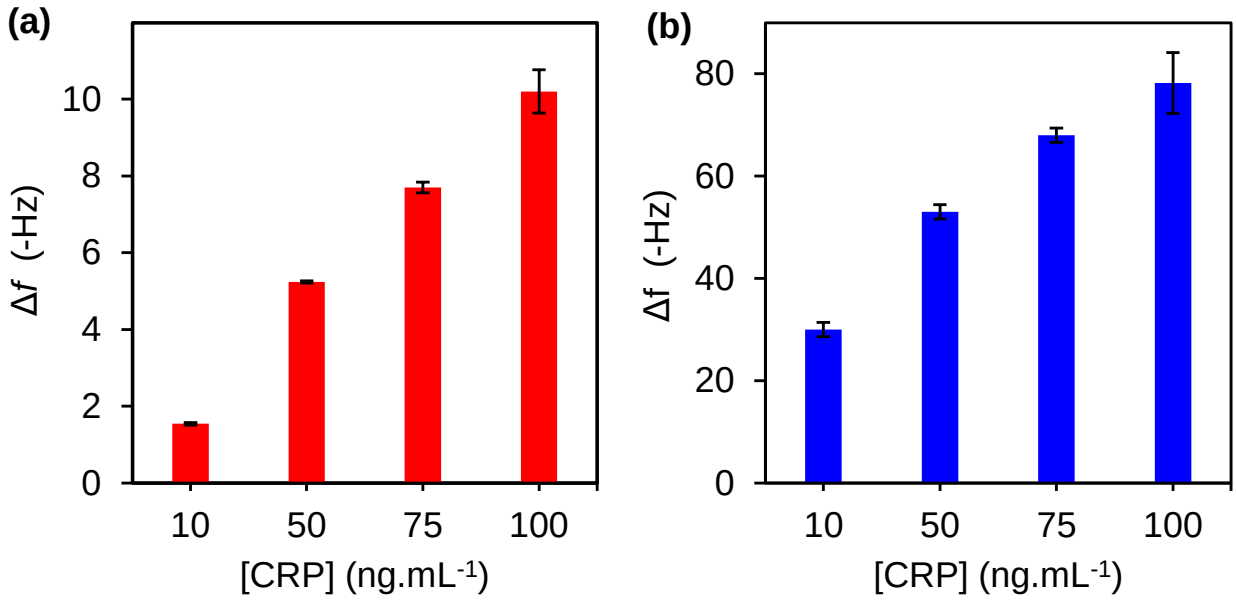


Fig. S3 Bar chart showing the reproducibility of the immunosensor for (a) direct CRP and (b) enhancement of CRP using *anti*-CRP-pAb at varying [CRP].

Table S2: Table showing the RSD for the reproducibility of the immunosensor for the direct CRP and enhancement of signal of the CRP using *anti*-CRP-pAb. (n = 3)

[CRP] (ng.mL^{-1})	direct CRP (RSD %)	Enhanced signal *anti*-CRP-pAb (RSD %)
10	1.83	4.71
50	0.54	2.67
75	1.84	2.08
100	5.54	7.59

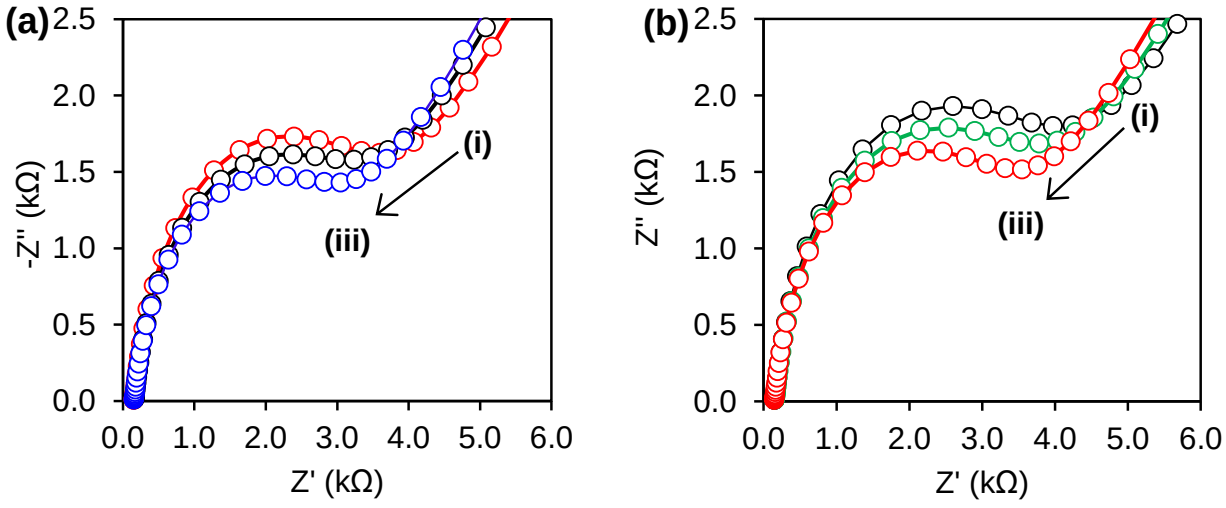


Fig. S4 Nyquist plots showing a decrease in charge transfer resistance (ΔR_{CT}) at higher CRP concentrations (i) 100 ng.mL^{-1}, (ii) 200 ng.mL^{-1} and (iii) 400 ng.mL^{-1} for (a) direct and (b) enhanced detection of CRP using *anti*-CRP-pAb.