Electronic Supplementary Information

An ultra-sensitive impedimetric immunosensor for detection of serum oncomarker CA-125 in ovarian cancer patients

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1. Synthesis of L-Cysteine-Capped CdSe QDs (L-Cys capped CdSe QDs):

Fig. S1 depicts different-sized L-Cys capped CdSe QDs synthesised in the aqueous media and characterised using absorption and fluorescence spectroscopy. The first exitonic absorption peak value (λ), indicated in the Uv-Vis absorption spectrum, was employed for determining the diameter of QDs using a previously reported empirical equation (1).[1] The calculated diameter was used for the determination of molar particle extinction coefficient (equation 2). Finally, the concentration of QD nanocrystals was estimated by Lambert-Beer's law (equation 3) in witch A (a.u.), ϵ (L/mol.cm), C (mol/L) and L (cm) are the absorbance at the first excitonic absorption peak, the extinction coefficient per mole of CdSe nanocrystals, the molar concentration of the QD nanocrystals, and the path length (1 cm) of the radiation beam respectively. During the synthesis of CdSe QDs, different-sized nanocrystals with tunable fluorescence emission were obtained through increasing the reflux time from 1 h to 8 h.

Equation (1):

$$D = (1.6122 \times 10^{-9})\lambda^4 (2.6575 \times 10^{-6})\lambda^3 + (1.6242 \times 10^{-3})\lambda^2 - (0.4277)\lambda + (41.57)$$

Equation (2): $\epsilon = 5857(D)^{2.65}$

Equation (3): $A = \epsilon CL$

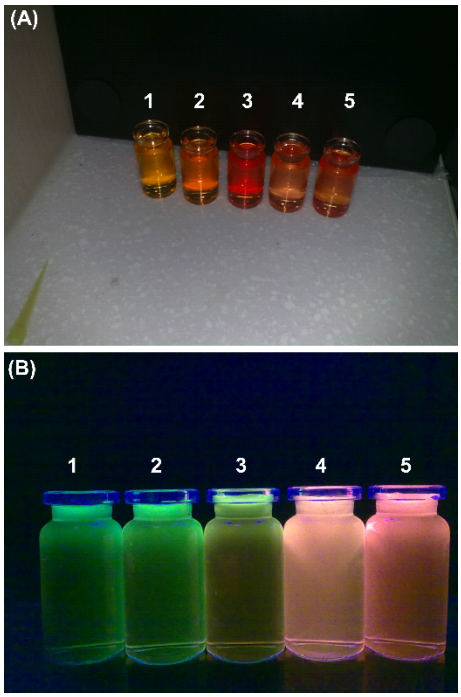

Fig. S1 Normal and fluorescent images of different-sized CdSe QDs capped with L-Cys. The synthesised QDs from various refluxing times (1:1h, 2:1.5, 3:2h, 4:4h, 5:8h) are shown under ambient light (A) and under 365 nm ultraviolet light (B). The emission wavelength red shifted when their sizes increased gradually.

2. The influence of the antigen-antibody incubation temperature on the CA125 biosensor

Fig. S2 shows that antigen-antibody complexation is affected by incubation temperature. Electrochemical impedance spectroscopy (EIS) was technically employed to study the response of the CA-125 immunosensor incubated under different temperatures. EIS technique is able to monitor faradaic impedance that indicates the interfacial electron transfer resistance (R_{et}) of the CA-125 biosensor. The complex of antigen-antibody formed on the surface of the CA-125 immunosensor (R_{et}) increased as a result of changing in the dielectric constant of the medium at the biosensor surface. Moreover, all CA-125 antigen-binding sites at the biosensor surface were fully occupied, and the faradaic response became fixed and reached plateau.

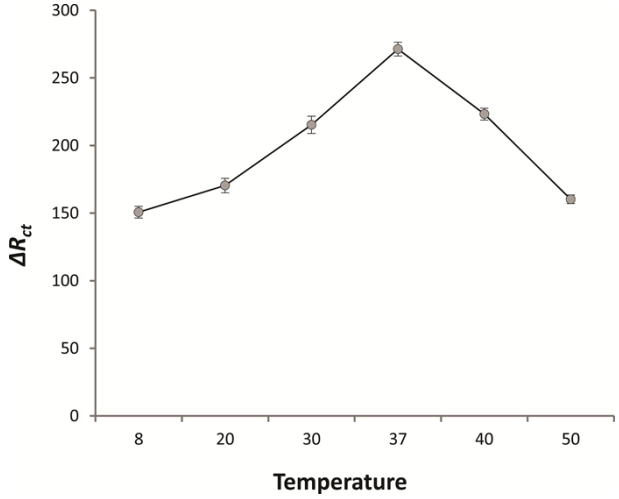

Fig. S2 The effect of antigen-antibody incubation temperature on the impedimetric response of the CA-125 biosensors. Experiments were performed using 50 mU/mL CA-125 antigen.

3. The influence of antibody-antigen incubation time on biosensor performance

EIS method is able to identify R_{et}, which is adapted during the antigen-antibody reaction.[2] Antigen-antibody reactions on the CA-125 biosensor surface were a time-dependent process that could be monitored by EIS indicating that the R_{et} reached to its own maximum value after 60 min (Fig. S3). This finding shows that all antigen binding sites on the biosensor surface are occupied after 60 min incubation time, which is considered as the optimum time for the complexation of the antigen with the CA-125 immunosensor.

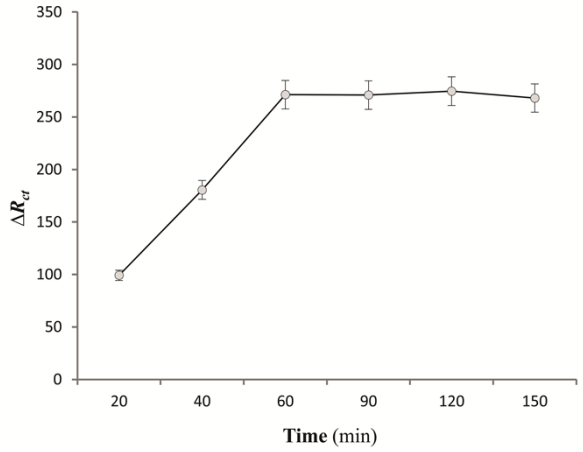

Fig. S3 The optimised antigen-antibody reaction time on the biosensor surface. Biosensor was immersed in the CA-125 antigen solution (50 mU/mL) at 37°C and examined by the EIS method at different time points (20, 40, 60, 90, 120, 150 min).

4. Stability assay for CA-125 biosensor

The stability tests were carried out every 3 days up to 3 weeks. The interfacial changes in physicochemical properties of the immunosensor were negligible and the relative EIS response of the biosensors during the stability assay showed less than 10% fluctuation (Fig. S4).

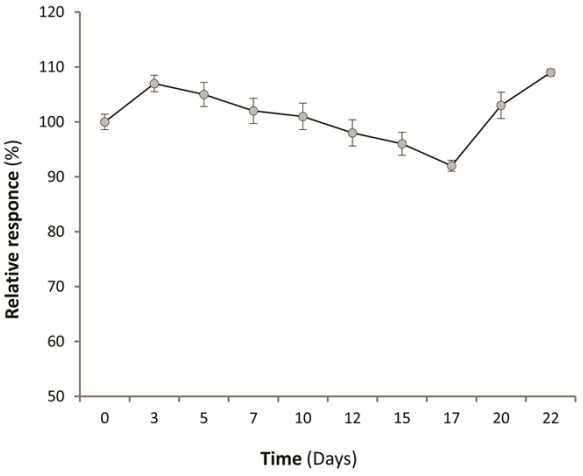

Fig. S4 The stability assay of the CA-125 immunosensor. The R_{et} values showed less than 10 % (n=5) deviation during the examination time period (3 weeks).

5. Calibration plot of CA-125 immunosensor

For calibration of the CA-125 immunosensor, the EIS response of various concentrations of the CA-125 antigen from 1 mU/mL to 10 U/mL was measured using Fe $(CN)_6^{3-/4-}$ in phosphate buffer solution. The linear range was between 0 and 100 mU/mL with detection limit of 1.6 mU/mL. The equation of the calibration curve was ΔR_{et} (Ω) = (4.849±0.24) x + 102.38±2.4 with R^2 = 0.9966 (Fig. S5). The ΔR_{et} represents the resistance differences before and after immersion of modified electrode in various concentrations of the antigen and x refers to the concentration of the CA-125 (mU/mL). Important analysed data are shown in Table S1.

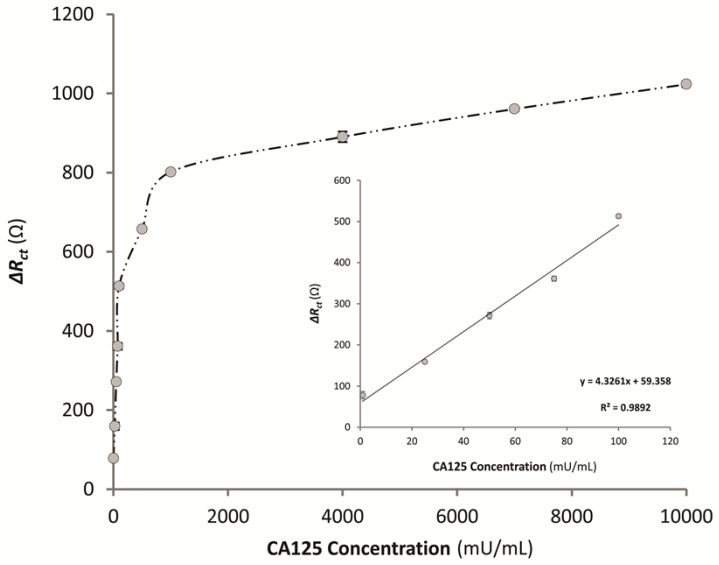

Fig. S5 The calibration plot of the CA-125 immunosensor. Inset represents a linear response attributed to the concentration of CA-125, increasing from 0 to 100 mU/mL.

Table S1 Equivalent circuit fit values for successive fabrication steps of the CA-125 immunosensor

Modification steps	R_s (Ω)		*CPE* (μF)		R_{ct} (Ω)		Z_w (Ω) ×10^{-4}		χ^2
	Value	EE%	Value	EE%	Value	EE%	Value	EE%	
Bare gold electrode	206.91	1.26	2.17	2.61	142.67	1.53	6.44	1.88	0.095
MPA modified gold electrode	237.80	1.10	1.69	2.04	2474.06	1.67	4.23	1.60	0.085
MPA|AuNPs@SiO_2 modified electrode	244.65	1.06	1.22	6.65	859.92	3.41	5.03	0.83	0.048
MPA|AuNPs@SiO_2|QD modified electrode	209.46	1.02	1.84	3.75	477.28	2.19	7.28	0.96	0.055
MPA|AuNPs@SiO_2|QD|mAb modified gold electrode	232.17	1.54	1.38	2.87	992.97	1.71	7.08	2.29	0.073

R_s: Solution resistance; *CPE*: Constant phase element; Z_w: Warburg Impedance; χ^2: Goodness of fit.

6. Recovery test

The recovery assessment of the CA-125 immunosensor was carried out by spiking serum samples with diverse concentrations of CA-125 (Table S2). The recovery of the electrode was calculated as percentage using the following equation:

$$R\% = \frac{(spiked\ sample\ result\ -\ unspiked\ sample\ result) * 100\%}{known\ spike\ added\ conccentration}$$

Table S2 Recovery analysis of the CA-125 specific immunosensor in healthy human samples

Healthy human samples	Initial detected (C2) CA125 (U/mL)	RSD% (n=5)	X-fold dilution	Added (C3) CA125 (mU/mL)	Analysed (C1) CA125 (U/mL)	Recovery %	RSD% (n=5)
S1	24.2	2.52	1000	0	24.1	-	-
S2	8.5	1.17	1000	25	31.3	91.2	1.53
S3	15.4	4.32	1000	50	64.5	98.2	1.68
S4	3.7	1.11	1000	75	79.1	100.5	0.94

RSD: Relative standard deviation; diluting buffer (5 mM; $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$) in PB, pH 7.0).

References

1 W. W. Yu, L. Qu, W. Guo and X. Peng, *Chem Mater*, 2003, 15, 2854-2860.

2 M. Saberian, H. Hamzeiy, A. Aghanejad and D. Asgari, *Bioimpacts*, 2011, 1, 31-36.