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Supplementary Information

Ultrasensitive electrochemical detection of prostate-specific antigen (PSA) using gold-coated magnetic nanoparticles as 'dispersible electrodes'

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S1. Experimental materials and methods

Materials

Chemical	Abbreviation	Purity	Supplier
Polyethylenimine, Branched, 25 kDa	PEI	-	Sigma-Aldrich, Sydney
Gold (III) chloride trihydate	$HAuCl_4.3H_2O$	>99.9%	Sigma-Aldrich, Sydney
DL-α-lipoic (thioctic acid)	-	-	Fluka, Sydney
N-(3-dimethylaminopropyl)-*N*'-ethyl-carbodiimide hydrochloride	EDC	98%	Sigma-Aldrich, Sydney
N-hydroxysuccinimide	NHS	98%	Sigma-Aldrich, Sydney
Monoclonal anti-PSA antibody specific to epitope 3 of PSA (capture antibody)	Ab_1	-	Abcam from Sapphire Bioscience, Sydney
Horseradish peroxidase (HRP) conjugated monoclonal anti-PSA antibody specific to epitope 1 of PSA (detection antibody)	Ab_2	-	Abcam from Sapphire Bioscience, Sydney
Ferrocenemethanol	-	97%	Sigma-Aldrich, Sydney

Instrumentation

Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) images were taken with a Philips CM 200 TEM operating at 200 kV and Hitachi s900 SEM respectively. A Thermo Scientific VG-ESCALAB 220-iXL spectrometer with a monochromated Al Kα X-ray source (1486.6 eV) was used for X-ray photoelectron spectroscopy (XPS) analysis. The spectra were recorded at a take-off angle of 90° and an analyser pass energy of 20 eV. Chronoamperometry measurements were performed using a

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microAutolab Type III potentiostat and the data was analysed by General Purpose Electrochemical System (GPES) version 4.9 software.

Synthesis of Ab_1-Au@MNPs

Au@MNPs used in this work were synthesized using the method previously developed by Goon *et al*. Briefly, iron oxide (Fe_3O_4) nanoparticles were synthesised by mixing 0.7 g of $FeSO_4$ in 80 mL of Milli-Q water, and subsequently the addition of 10 mL of 2.0 M KNO_3 and 10 mL of 1.0 M NaOH in an oxygen-free environment. The $Fe(OH)_2$ formed initially was heated to 90°C for 2 hours, allowing the oxidation of $Fe(OH)_2$ to form Fe_3O_4 nanoparticles. The Fe_3O_4 nanoparticles were magnetically separated from the mixture using a magnet and the remaining solution discarded. The Fe_3O_4 nanoparticles collected were rinsed 3 times with MilliQ water followed by the addition of 20 mL of 20 g/L polyethylenimine (PEI) solution. The mixture was sonicated for 2 min with an ultrasonic probe (Misonix S3000 Sonicator) and allowed to incubate at 90°C for 4 hours. The PEI-coated Fe_3O_4 nanoparticles were then rinsed with MilliQ water 3 times and resuspended in 25 mL of MilliQ water. Next, 2 nm colloidal gold nanoparticles were produced by the reduction of 1% w/w $HAuCl_4$ in 90 mL of Milli-Q water by 0.075% $NaBH_4$ in 38.8 mM sodium citrate solution. The PEI-coated Fe_3O_4 nanoparticle solution previously prepared was sonicated for 2 min and added to 90 mL of the Au colloid solution. The mixture was allowed to stir at 400 rpm for 4 hours at room temperature. The AuNPs-seeded Fe_3O_4 nanoparticles were magnetically separated from the excess Au colloid solution and rinsed 3 times with MilliQ water. AuNPs-seeded PEI-coated Fe_3O_4 nanoparticles were then added to 20 mL of 5 g/L PEI solution. The mixture was again sonicated for 2 min and allowed to incubate at 90°C for 4 hours. These PEI-coated AuNPs-seeded Fe_3O_4 nanoparticles were then magnetically separated from the solution, rinsed 3 times with MilliQ water and resuspended in 20 mL of MilliQ water. Full gold coating on the AuNPs-seeded Fe_3O_4 nanoparticles was performed *via* three iterations of $HAuCl_4$ reduction. Firstly, 20 mL of the PEI-coated AuNPs-seeded Fe_3O_4 nanoparticle solution was sonicated for 2 min and 10 mL of the nanoparticle solution was added into 110 mL of 0.01 M NaOH solution (pH ~11.5). The mixture was stirred mechanically throughout the entire gold-coating process at 300 rpm. 0.725 mL of 1% $HAuCl_4$ was added to the mixture followed by the addition of 0.625 mL $NH_2OH.HCl$ 5 min after. For the next two iterations, 0.5 mL of 1% $HAuCl_4$ and 0.281 mL of $NH_2OH.HCl$ were added at

5 min intervals. The gold-coated magnetic nanoparticles (Au@MNPs) were magnetically separated from the solution, rinsed 3 times with MilliQ water and resuspended in 10 mL of MilliQ water.[1]

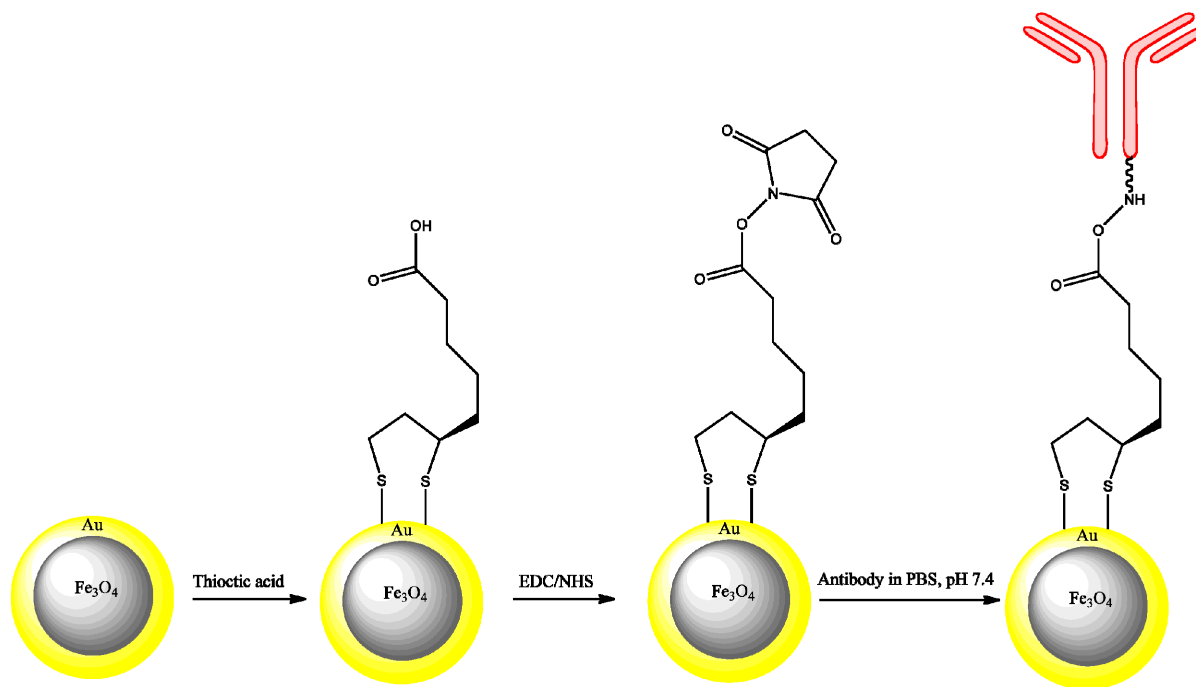

Figure S1: Outline of the modification process used for immobilization of Ab_1 onto Au@MNP.

Subsequently, the gold surface of Au@MNPs was modified with thioctic acid self-assembled monolayer (SAM) followed by tethering of monoclonal anti-PSA capture antibody (Ab_1) (Abcam) onto the thioctic acid SAM (Figure S1). Briefly, a 1 mL aliquot of the 10 mM thioctic acid solution was added into 10 mL of Au@MNPs solution. The mixture was sonicated for 2 min and allowed to incubate for 4 hours at room temperature. Next, the Au@MNPs were magnetically separated and rinsed 3 times with 75% ethanol and 25% water solution and subsequently with acetone. A stream of nitrogen gas was passed through the nanoparticles to facilitate the drying process. The mass of the thioctic acid modified Au@MNPs was then obtained using an analytical balance. The thioctic acid modified Au@MNPs were first rinsed with 75% ethanol and 25% water. 1 mL of 10 mM EDC and 10mM NHS in 75% ethanol and 25% water solution was added to the Au@MNPs and allowed to incubate for 1 hour at room temperature. The EDC/NHS-activated thioctic acid modified Au@MNPs were magnetically separated and rinsed 3 times with 75% ethanol and 25% water and then rinsed once with phosphate buffer saline (PBS) pH 7.4 followed by the addition of 500 μL of 100 μg/mL Ab_1 in PBS. The Au@MNPs were dispersed in solution

using a vortex mixer and allowed to incubate overnight at 4°C. The Ab_1-functionalised Au@MNPs (Ab_1-Au@MNPs) were magnetically separated and rinsed 3 times with PBS. Using the mass of Au@MNPs measured previously, the Ab_1-Au@MNPs were then dispersed in an appropriate amount of PBS such that the concentration of the Ab_1-Au@MNPs is 8 g/L.

Electrochemical detection of PSA *via* sandwich ELISA strategy

The Ab_1-Au@MNPs were used to capture and electrochemically detect standard solutions of PSA analyte in PBS. First, 50 μL of the 8 g/L Ab_1-Au@MNPs solution were added to a 50 μL standard PSA solution of 2 times (2X) concentration. The Ab_1-Au@MNPs were dispersed in solution using a vortex mixer and allowed to incubate for 2 hours at room temperature. Next, the Ab_1-Au@MNPs exposed to PSA were magnetically separated and rinsed 3 times with PBS. 50 μL of 100 μg/mL HRP-conjugated anti-PSA detection antibody (Ab_2) in PBS was added to the Au@MNPs. The Au@MNPs were then dispersed in solution using a vortex mixer and allowed to incubate for another 2 hours at room temperature. After that, the Au@MNPs were again magnetically separated, rinsed 3 times with PBS and resuspended in 4 mL of 1mM ferrocenemethanol in PBS solution.

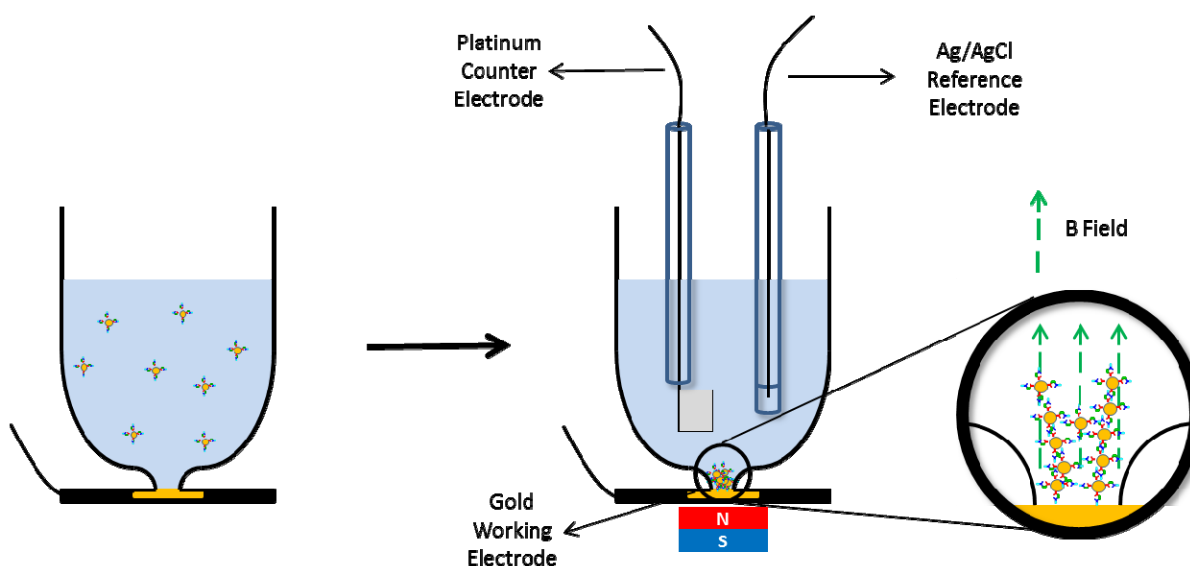

Figure S2: Schematic illustration of the electrochemical cell setup used for electrochemical analysis of Au@MNPs. The anti-PSA capture antibody functionalised Au@MNPs were dispersed in the sample solution and then a magnet was used to attract the Au@MNPs to the gold working electrode surface.

A custom glass electrochemical cell setup (Figure S2) was used for all electrochemical analysis experiments involving Au@MNPs. A 1 cm x 1 cm gold foil was first rinsed with piranha solution and then polished with 1.0, followed by 0.5 and 0.05 µm alumina/water slurry for 3 minutes each on microcloth pads prior to use in the electrochemical cell. Trace alumina was removed from the gold electrode surface by rinsing with Milli-Q water. The polished gold foil was then assembled into the electrochemical cell and connected to the working electrode lead of a potentiostat as shown in Figure S1. The Au@MNPs in ferrocenemethanol solution was added into the electrochemical cell. A magnetic field was applied at the base of the cell for 5 min using a neodymium disc magnet in order to attract Au@MNPs to the gold working electrode. 0.2 mL of 10 mM H_2O_2 was later added as a substrate for the reaction catalyzed by HRP. Subsequently, the platinum flag counter electrode and Ag/AgCl reference electrode were immersed in the electrochemical cell solution and chronoamperometry measurements were performed *via* a potentiostat. Multiple samples of Ab_1-AuNPs added to a range of standard PSA solutions with different 2X concentrations were prepared. The current recorded at t = 200 s were used for construction of a calibration curve.

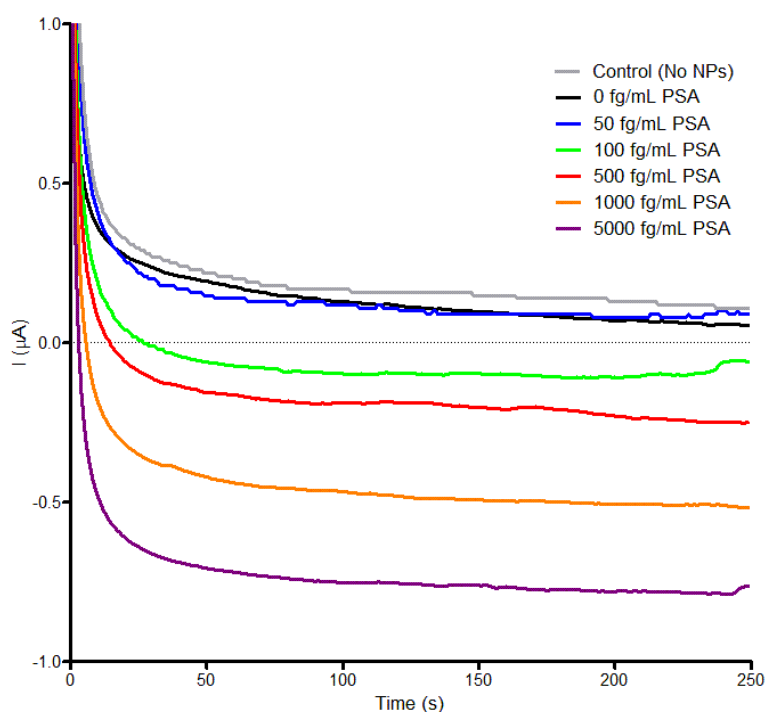

Figure S3: A chronoamperometry plot indicating the current response of various PSA standard samples at a potential of +0.15 V versus Ag/AgCl.

S3. X-ray photoelectron spectroscopy (XPS) characterization evidence for surface modification of Au@MNPs

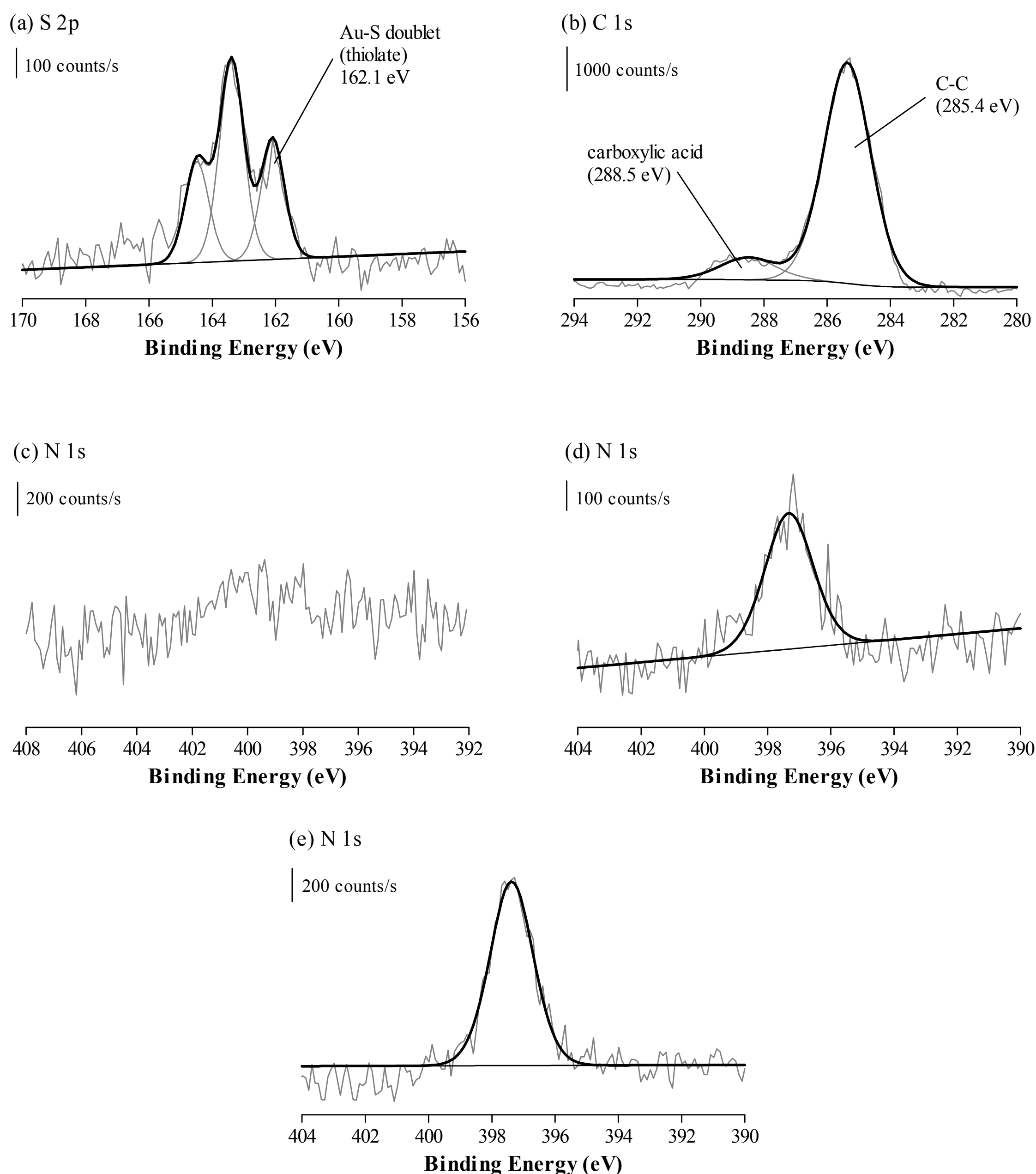

Figure S4: XPS spectra of Ab_1-Au@MNPs taken at various stages during the surface modification process. (a) S 2p region after self-assembly of thioctic acid; (b) C 1s region after self-assembly of thioctic acid; (c) N 1s region after self-assembly of thioctic acid; (d) N 1s region after EDC and NHS activation; (e) N 1s region after attachment of Ab_1.

The presence of thioctic acid self-assembled monolayer on the Au@MNPs after surface modification is evidenced by the occurrence of several characteristic peaks in the high-resolution XPS spectra of the S 2p and C 1s regions (Figure S4a and S4b). Triplet peaks were

observed in the S 2p region occurring at 162.08, 163.38 and 164.48 eV which are attributed to thioctic acid molecules chemisorbed onto gold.[2] In addition, the peaks at 285.4 and 288.5 eV in the C 1s region which correspond to the presence of methylene and carboxylic groups respectively further support the successful surface modification of Au@MNPs with thioctic acid. Activation of the carboxylic group in thioctic acid by EDC and NHS results in the emergence of a peak in the N 1s region of the spectra (Figure S4d) which was not present previously (Figure S4c). Subsequently, the attachment of Ab_1 onto the Au@MNPs is confirmed by the increased signal counts of the 397.3 eV peak in the N 1s region (Figure S4e) relative to the same peak observed before incubation of Au@MNPs with Ab_1 (Figure S4d).

S4: Scanning electron microscopy (SEM) characterization of magnetically assembled Au@MNPs

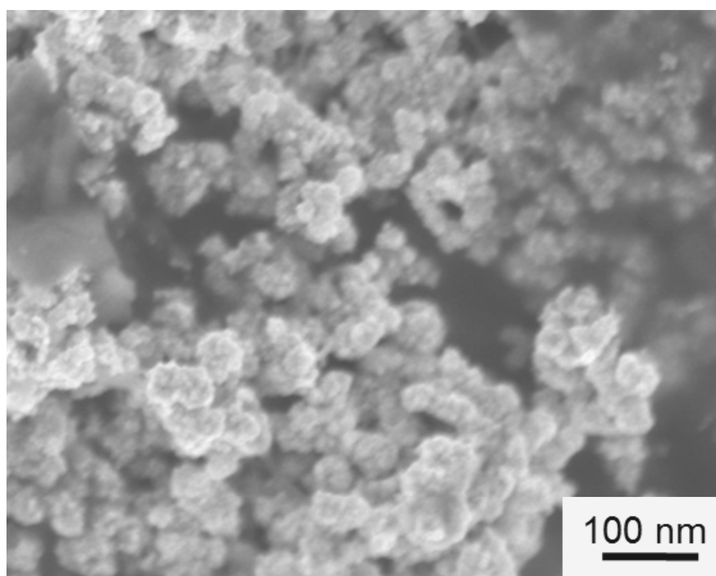

Figure S5: SEM image of magnetically assembled Au@MNPs shows the packing of Au@MNPs on gold electrode.

S5. Relationship between the measured reduction current and incubation time of Ab_1-Au@MNPs with PSA and PSA- Ab_1-Au@MNPs with Ab_2.

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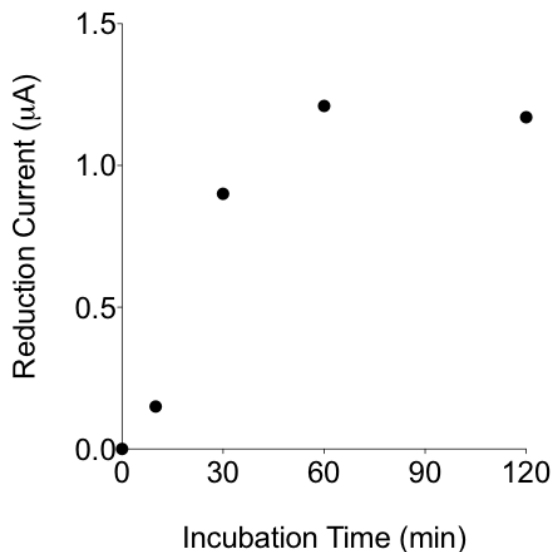

Figure S6: A plot of the reduction current measured from Ab_2-PSA-Ab_1-Au@MNPs at a potential of +0.15 V versus Ag/AgCl as a function of the incubation time of Ab_1-Au@MNPs in 5 pg/mL PSA in PBS and subsequently PSA-Ab_1-Au@MNPs in 100 μg/mL Ab_2 in PBS.

Figure S6 above illustrates the increase of the measured reduction current from Ab_2-PSA-Ab_1-Au@MNPs as the incubation time increases. The reduction current saturates after 60 min of incubation time suggests that the performance of Ab_1-Au@MNPs electrochemical immunosensor is limited by the antibody-antigen binding kinetics between Ab_1 and PSA, and, PSA and Ab_2.

References

1. I. Y. Goon, L. M. H. Lai, M. Lim, P. Munroe, J. J. Gooding and R. Amal, *Chemistry of Materials*, 2009, **21**, 673-681.
2. E. Chow, T. R. Gengenbach, L. Wieczorek and B. Raguse, *Sens. Actuator B-Chem.*, 2010, **143**, 704-711.