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Detection of Sub-Femtomolar DNA Based on Double Potential Electrodeposition of Electrocatalytic Platinum Nanoparticles

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Details of Experimental Procedures

Materials.

The buffer and dodecanethiol were obtained from Sigma Aldrich. The buffer, denoted as 1 M NaCl-TE, contained 1.0 M NaCl, 10 mM Tris-HCl, and 1 mM ethylenediaminetetraacetic acid (adjusted to pH 7.0 by adding 1.0 M NaOH) and was used for DNA hybridization. Platinum nanoparticles (diameter 50-70 nm) for uniform functionalization with probe strand DNA were purchased from Particular GmbH. All aqueous solutions were prepared using Milli-Q water. The oligonucleotides were purchased from Eurogentec©™ and their purity was >98%. The base sequences are as follows:

Capture: 5'- CGG-CAG-TGT-TTA-TCA -3' – SH

Target: 5'-TGA-TAA-ACA-CTG-CCG-TTT-GAA-GTC-TGT-TTA-GAA-GAA-ACT-TA-3'

The target sequence occurs in the ribosomal DNA of the strain of the specific S. aureus pathogen that causes mastitis (mammary gland inflammation).

Probe: SH-5'- AT-AGT-TTC-TTC-TAA-ACA-GAC- -3'

1 Base Mismatch: 5'-TGC-TAA-ACA-CTG-CCG-TTT-GAA-GTC-TGT-TTA-AAA-GAA-ACT-TA-3'

3 Base Mismatch: 5'-TGC-TAA-ACA-CTG-CCG-CTT-GAA-GTC-TGT-TTA-GAT-GAA- ATA-TA-3'

Instrumentation.

A three-electrode electrochemical cell was used at a temperature of 22±2°C. The working electrode was a 2 mm radius planar gold disc. It was polished with a nylon cloth with 1 µm diamond polish and thoroughly rinsed with Milli-Q water and ethanol before sonication in Milli-Q water for 5 minutes. Voltammetry in acid was used to determine the surface roughness factor by scanning the electrode between +1.500 and -0.300 V. The counter electrode was a large area coiled platinum wire and a silver/silver chloride (Ag/AgCl in 3 M KCl) acted as reference. Dynamic Light Scattering (DLS) experiments were performed at 25 °C on a Nanosizer NanoZS (Malvern Instruments, Malvern U.K.) using a detection angle of 173° and a 3 mW He–Ne laser was operating at a wavelength of 633 nm. The dhyd values reported are the Z-average diameters (mean hydrodynamic diameter based upon the intensity of scattered light). The polydispersity indices were also calculated from the Cumulants analysis as defined in ISO13321.[18] The intensity size distributions were obtained from analysis of the correlation functions using the Multiple Narrow Modes algorithm based upon a non-negative least-squares fit[19] using Dispersion Technology software (v. 5.3, Malvern Instruments; Worcestershire, U.K.). The values used for the viscosity of platinum in 0.01 M H_2SO_4 was 26.7 cPa·s at 298 K.

Monolayer self assembly.

The gold electrodes were cleaned by placing them in piranha solution (3:1 mixture of sulphuric acid and 30% hydrogen peroxide, Caution, this mixture reacts violently with organic material!) for 20 min, followed by thorough rinsing with ultra pure water. The electrode was then scanned between +1.500 and -0.300 V in 0.01 M H_2SO_4 to measure the surface roughness of the gold electrode and to ensure that the electrode is electrochemically clean. Afterwards, the gold disk electrode was washed with ethanol and then placed in a 1 mM solution of dodecanethiol in ethanol and monolayer self-assembly allowed to proceed for 7 hours. After the formation of the monolayer, the substrate was rinsed 4 to 5 times with ethanol and dried under a N_2 stream.

Double pulse deposition

Platinum nanoparticles, PtNPs, were electrodeposited from 1 mM hydrogen hexachlorideplatinate (IV) hydrate (H_2PtCl_6) in 0.5 M H_2SO_4 using the defects within the self-assembled monolayer as templates. A 20 ms nucleation pulse was applied to the gold disc electrode at a potential of -1600 mV followed by a growth pulse at – 200 mV for 60 s. Following nanoparticle deposition the electrode was removed from the electrolyte, rinsed with Milli-Q water and dried under a stream of high purity argon

DNA Probe Immobilization and Hybridization.

Step 1: Regio Selectively DNA Functionalized Platinum Nanoparticles. The PtNPs were functionalized with probe oligo (5' thiolate) by immersing the nanoparticle functionalized electrode in a 10 µM solution of the probe DNA strand dissolved in 1 M NaCl-TE Buffer for 2 hours. The modified electrodes were then washed with deionized water for 15 s to remove

loosely bound oligo and immersed in 0.01 M H_2SO_4. The oligo functionalized platinum nanoparticles were then desorbed by applying a current of +0.01 A (resulting potential is negative) for 120 seconds.

Step 2A: Monolayer of Capture Strand DNA. A monolayer of capture strand DNA was assembled on a freshly polished and electrochemically cleaned gold disk electrode by immersing it in a 10 µM solution of the capture strand DNA dissolved in 1 M NaCl-TE Buffer. After 5 hours, the electrode was rinsed with deionized water for 15 s to remove loosely bound oligo.

Step 2B: Hybridization of target oligo to the capture surface. Hybridization of the target to the immobilized capture strand was performed at 37°C in 1 M NaCl-TE Buffer for 90 min. Following hybridization, the modified electrode was rinsed thoroughly with buffer.

Step 2C: Probe Hybridization. The nanoparticle labelled probe DNA was then hybridized to the complementary section of the target not used for binding to the capture strand for 2 hours at 37 °C in 1 M NaCl-TE Buffer. Finally, before quantitation, it was thoroughly washed with deionized water.

Electrochemical detection of S. aureus ss-DNA target.

Following assembly of the capture-target-nanoparticle labelled probe DNA sequence, the modified electrode was placed in an aqueous solution of 0.01 M H_2SO_4 and the current measured at -0.250 V after equilibration for approximately 10 minutes. Then, sufficient hydrogen peroxide added to give a final concentration of 2 mM and the reduction current associated with peroxide reduction at the bound PtNPs measured at -0.250 V after 40 minutes. The analytical response is taken as the difference in current, Δi, measured before and after peroxide addition.

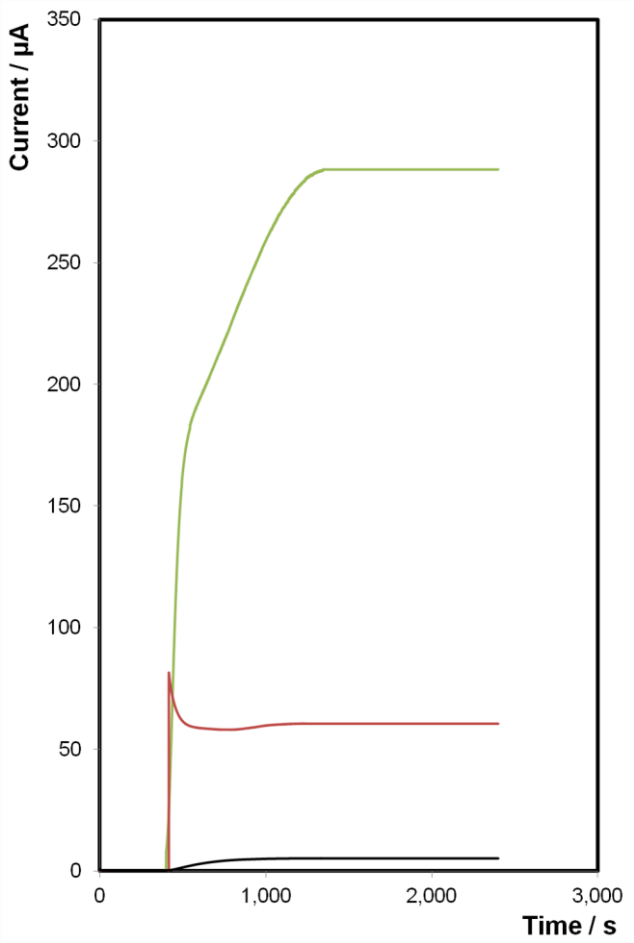

Figure S1 Current–time curves denote responses for nanoparticles regioselectively modified with probe DNA (green line), with non-complementary tDNA hybridised (red line) and

Concentration		Log DNA	i before H2O2	i after H2O2	Difference in current (i)	mA (i)	Average of 3 runs	Std Deviation
1 µM tDNA (fully Complementary)								
1 µM	1.00E-06	-6	1.05E-06	2.85E-03	2.85E-03	2.85	2.84	0.094037268
1 µM	1.00E-06	-6	2.63E-06	2.75E-03	2.75E-03	2.75		
1 µM	1.00E-06	-6	4.24E-06	2.94E-03	2.94E-03	2.94		
1 µM tDNA (1 base mismatch)								
1 µM	1.00E-06	-6	1.41E-07	6.06E-04	6.06E-04	0.61	0.60	0.048867413
1 µM	1.00E-06	-6	1.42E-06	6.44E-04	6.43E-04	0.64		
1 µM	1.00E-06	-6	2.52E-07	6.39E-04	6.39E-04	0.64		
1 µM tDNA (3 base mismatch)								
1 µM	1.00E-06	-6	1.18E-07	3.19E-06	3.07E-06	0.00307	0.00321	0.000366648
1 µM	1.00E-06	-6	1.27E-07	5.47E-06	5.34E-06	0.00534		
1 µM	1.00E-06	-6	1.33E-06	1.23E-06	1.22E-06	0.00122		

where the probe strand is not labelled with a nanoparticle (black line). In all cases, the supporting electrolyte is 0.01 M H_2SO_4. The applied potential is -0.250 V. At 400 s, sufficient H_2O_2 was added to the cell to give a 200 mM solution.

Table S1 Statistical Information for the electrochemical detection of *Staph. Aureus* mastitis DNA on a 2 mm diameter bare electrode following hybridization with probe DNA that is labeled with PtNPs.