Electronic Supplementary Information (ESI)

Regio Selective Functionalisation of Gold Nanoparticles with DNA

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1 Details of Experimental Procedures

1. Materials.

1mM dodecanethiol was 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 solution DNA probe assembly. All aqueous solutions were prepared using doubly distilled water. The oligonucleotides were purchased from Eurogene® and their purity was >98%. The base sequences are as follows:

Capture: 5’-ACG-GCA-GTG-TTT-AGC-3’ – SH
Target: 5’-TGA-TAA-ACA-CTG-CCG-TTT-GAA-GTC-TGT-TTA-GAA-GAA-ACT-TA-3’
Probe: 5’ Horseradish peroxidase – AAG-CTT-CTA-AAC-AGA-CT-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’

2. Instrumentation.

A three-electrode electrochemical cell was used throughout at a temperature of 22±2°C. The working electrode was a 2 mm diameter planar gold disc. It was polished with a nylon cloth with 1µM 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. The counter electrode was a large area coated platinum wire and a saturated silver/silver chloride (Ag/AgCl) acted as reference.

Infrared attenuated total reflection (ATR) spectra were recorded on Varian 610IR microscope. Fourier transform infrared (FT-IR) microscopy was set at a resolution of 4 cm⁻¹ measuring IR reflection (transmission) with a 15x reflection objective. Spectral range is from 700 to 3700 cm⁻¹. Spectra were run in a sample compartment flushed for 20 min with dry air. They were referenced to a background spectrum previously recorded on the crystal without the nanoparticles and cleaned under the same conditions as for the covered crystal. Two kinds of analysis were carried out. 1mM C₆₁₂ which were allowed to self assemble for different corresponding times (1-10 hours) on a thermally annealed, vapour deposited gold on silicon, substrate. The second characterisation was carried for each SAM of C₆₁₂ with different electrodeposition times of silver nanoparticles ranging from 30 seconds to 3 minutes. Atomic force microscopy (AFM) images were recorded on a Veeco Bioscope II in contact mode using commercial Si₃N₄ cantilever tips. These tips are pyramidal in shape with spring constants between 0.04 and 0.08 N/m, and tip sizes were normally less than 20 nm radius. The AFM imaging was carried out under dry conditions.

3. Experimental

Monolayer self assembly

Pirahna solution is used to remove organic residue from substrates. CAUTION! Pirahna solution reacts violently with organic materials. The gold electrodes (2 mm in diameter) were soaked in a 3 : 1 mixture of sulphuric acid and 30% hydrogen peroxide for 20 min, followed by a thorough rinse with ultra pure water. After that, the electrode was scanned between 0 and 1.25V in 0.1 M H₂SO₄ to measure the surface roughness of the gold electrode. Afterwards the gold disk electrode was washed with ethanol and then placed in a 1 mM dodecanethiol monolayer (12 carbons with a thiol on the end) made up in ethanol. The monolayer was allowed to self assemble for a given amount of time (1-10 hours). After the formation of the monolayer, the substrate was rinsed 4 to 5 times with ethanol and dried under a N₂ flow.

Construction of nanogold working electrode

Current-time transients were used for studying the nucleation and growth of gold nanoparticles onto a gold disc electrode. A 3 mM HAuCl₄ solution was used to deposit gold onto the working electrode by applying a fixed potential of -0.273 V for 3 min.

DNA probe immobilisation and hybridisation.

Step 1: A monolayer of the capture oligo (3’ thiolate) was prepared by immersing the working electrode (unmodified, SAM modified or SAM-AuNP modified) in a 1µM oligo solution
prepared in 1M NaCl Buffer. After 5 hours the electrode was thoroughly washed with deionised water to remove any physisorbed material. Step 2: Hybridization of target oligo to the capture surface was performed at 37°C using a 150 pM oligo in hybridization buffer for 90 min. Following hybridization, the modified electrode was rinsed thoroughly with buffer. Step 3: The HRP-labelled probe oligo was hybridized to the target by immersing the modified electrode in a 1µM solution of the enzyme labelled oligo for 90 min at 37 °C. Finally, it was thoroughly rinsed and dried in a nitrogen stream.

Desorption of gold nanoparticles and electrochemical detection of Staph. Aureus ss-DNA target.

The functionalised AuNPs were stripped from the electrodes in phosphate buffer saline (0.1M KCl) by applying a current of 200 mA cm⁻² for 2 seconds. Two studies were carried out in which a bare electrode was placed in the suspended nanoparticle solution and the stripped electrode was placed into a fresh solution of phosphate buffer saline (0.1M KCl) for analysis. 1.81 mM hydroquinone was added to both systems and allowed to thoroughly deoxygenated using argon. Due to the sensitivity of the hydroquinone to photodegradation, the solution was prepared daily and the cell was wrapped in tinfoil to prevent any photochemical degradation. Cyclic voltammetry revealed that the hydroquinone is reduced to benzoquinone at ~ -0.2V. Chronoamperometry experiments were performed at the identified potential against Ag/AgCl electrode.