Supporting Information for:

Biofouling-Resilient Nanoporous Gold Electrodes for DNA Sensing

Pallavi Daggumati, Zimple Matharu, Ling Wang, Erkin Seker*

Department of Electrical and Computer Engineering, University of California, Davis, CA, 95616
*Corresponding author: eseker@ucdavis.edu

Supporting Information: Table of Contents

Fabrication of np-Au electrodes

Np-Au gold films were prepared by sputter deposition and subsequent dealloying. Briefly, glass cover slips were cleaned in piranha solution, composed of 1:4 volumetric ratio of hydrogen peroxide (30%) to sulfuric acid (96%), rinsed in deionized (DI) water, and dried under nitrogen flow prior to metal deposition. Metal deposition was carried out using a magneto-sputtering system (Kurt J. Lesker). First, a 160 nm-thick chrome layer was sputtered at 300 W to promote adhesion between glass and the subsequent metallic layers. Next, 80 nm-thick seed layer of gold was sputtered at 400 W and finally silver and gold were co-sputtered at 100 W and 200 W respectively to obtain a 600 nm-thick alloy layer. All depositions were performed successively under argon ambient at 10 mTorr. The composition of the alloy was 64% Ag and 36% Au (at. %) as determined by X-ray energy dispersive spectroscopy (EDS), (Oxford Instruments). The samples were then dealloyed in 70% nitric acid at 55 °C for 15 minutes to produce the np-Au films and then rinsed with DI water. The residual silver in np-Au samples after dealloying was estimated to be ~8% (atomic %) via EDS. Planar gold (pl-Au) electrodes were also fabricated by sputter-depositing a 50 nm-thick chrome adhesion layer followed by 250 nm-thick gold film onto piranha-cleaned glass cover slips.

Morphological characterization

Top and cross-sectional images of unannealed and annealed np-Au electrode with different morphologies were captured via scanning electron microscope (FEI Nova NanoSEM430) at 100 kX magnification. Top-view images of samples (image size: 2.5 µm X 3 µm) were analyzed using a custom automated analysis tool written in python based on openCV (http://opencv.org/) in order to determine the key features of various morphologies such as pore radii and pore areas. This analysis resulted in a distribution in pore sizes as shown in Figure S1. The median pore radius of unannealed np-Au was 14 nm and 30 nm in the case of annealed np-Au electrodes.

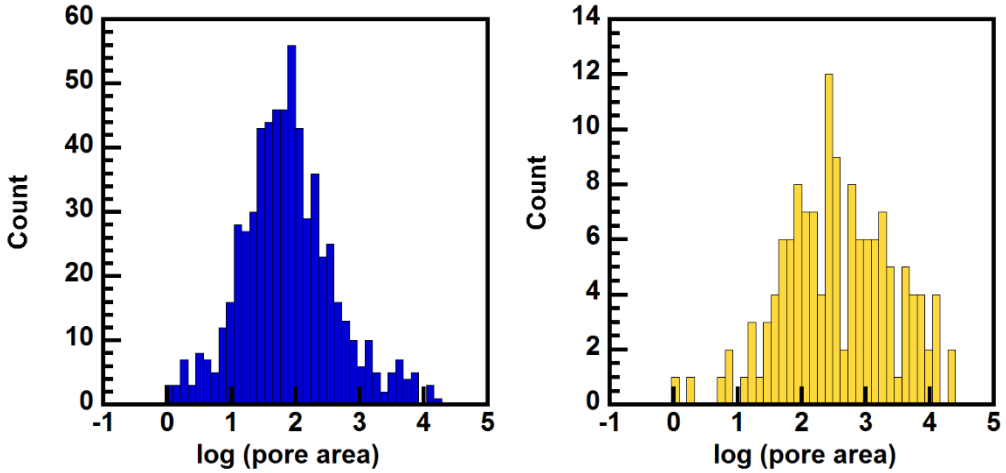


Figure S1: Pore size (nm^2) distributions of unannealed np-Au (left) and annealed np-Au (right). A wider spread in pore sizes is seen for annealed np-Au electrodes.

Electrochemical surface area characterization

The surface area of np-Au and planar Au electrodes was estimated electrochemically. Cyclic voltammetric (CV) measurements were performed in 0.05 M sulfuric acid at a scan rate of 50 mV/s over a potential range of -0.25 mV to 1.75 mV. Figure S2 illustrates the cyclic voltammograms of pl-Au and np-Au films. The electrical charge under the gold oxide reduction peak between the potentials 720 mV and 970 mV was converted into the effective surface area by using 450 $\mu C/cm^2$ as the specific charge required for gold oxide reduction. The ratio of the effective surface areas of different np-Au samples to the effective surface area of control planar Au sample was defined as *enhancement factor*, E_h. The effective surface area of np-Au samples was 6.55 cm^2 displaying a significant E_h of 10. Thermally-annealed np-Au samples had a lower E_h, 2.41 possibly due to pore coalescence resulting in a decrease in the number of pores.

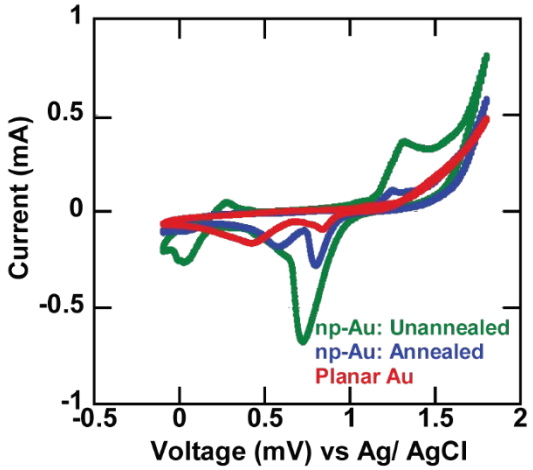


Figure S2: Cyclic voltammograms of unannealed and annealed np-Au, planar Au electrodes in 50 mM sulfuric acid. The surface area available is estimated using the charge under the gold oxide reduction peak.

Redox marker performance in presence of macro molecules

Cyclic voltammograms (CV) were acquired at a scan rate of 50 mV/s in response to potassium ferri/ferro cyanide complex ($[Fe(CN)_6]^{3-/4-}$). In the absence of BSA, both np-Au and planar Au electrodes displayed CVs characteristic of a diffusion controlled reaction of ferri/ferro complex. The electrodes were then incubated with electrolyte containing BSA and potassium ferro/ferricyanide prepared in 25 mM phosphate buffer (PB), pH: 7.4. The electrodes were interrogated every 5 minutes initially and intervals of 10 minutes later. The redox signal from unannealed np-Au electrode was stable (Figure S3a) while on pl-Au, the redox signal depleted by ~20% within 10 minutes and a total reduction of ~ 30 % was observed in 1 hour as shown in Figure S3 b & d. Also, an increase in the difference between redox peak potentials (peak separation) was observed on pl-Au over time (Figure S3c). This is also attributed to the BSA adsorption on the electrode surface. A slight increase in peak current is observed in over time (~6%) on np-Au electrodes. The permeation of redox markers bypassing the biofouled surface may explain such an increase in the np-Au signal observed over time.

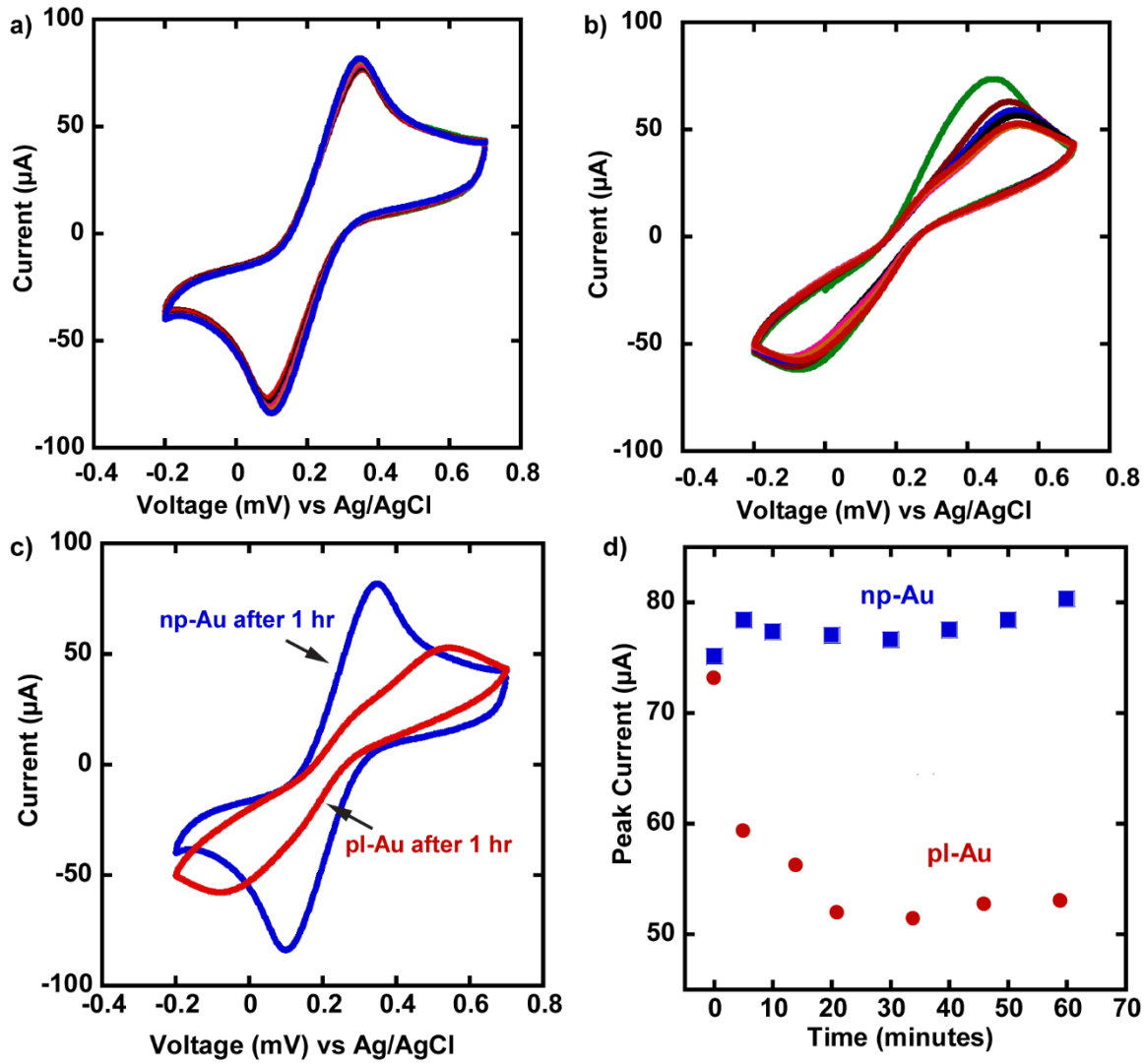


Figure S3: Cyclic voltammograms of (a) np-Au and pl-Au (b) in potassium ferri/ferro cyanide (5 mM) containing 2 mg/ml BSA prepared in PB (pH: 7.4) monitored for 60 minutes. (c) Comparison of np-Au and planar Au response after 1 hour. Drop in peak current and peak spreading is observed on planar Au. (d) Peak current observed over time for np-Au and planar Au electrodes. There is minimal change in peak current over time for np-Au.

DNA sensor preparation

The oligonucleotides used in this project consisted of 26 bases and were purchased from Integrated DNA Technologies, USA. The 5`end of ssDNA probe was modified with a C6 linker and thiol group. The sequences used were:

Probe ssDNA: 5ThioMC6-D/CGT GTT ATA AAA TGT AAT TTG GAA TT;
Target DNA: AAT TCC AAA TTA CAT TTT ATA ACA CG

Scheme 1 includes a graphical illustration of sensor preparation. Prior to DNA modification, the electrodes were first cleaned in dilute (1:4) piranha solution for 20s. These electrodes were then incubated with an immobilization solution containing 25 mM phosphate buffer (PB), 2 µM thiolated probe DNA and 50 mM $MgCl_2$ for 15 hours at room temperature. This was followed by

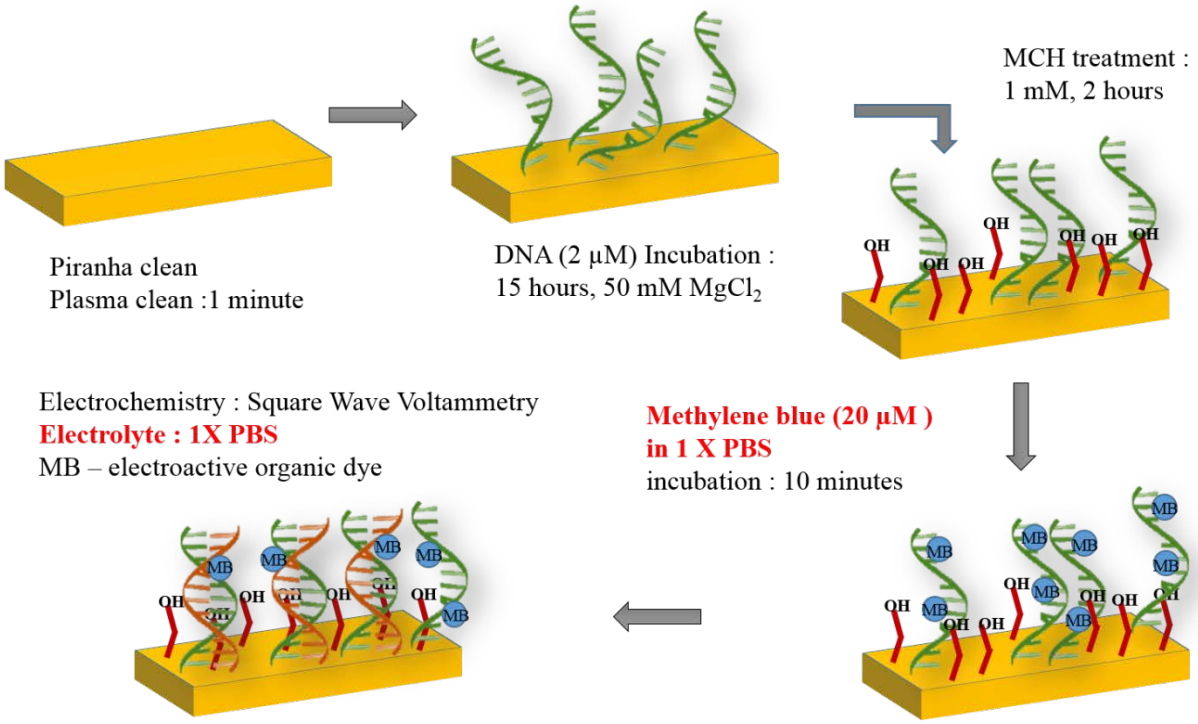
incubation with back-fill agent, 1 mM mercaptohexanol (MCH) prepared in PB for 2 hours to passivate the surface that has not been covered by probe DNA. The electrodes were thoroughly rinsed with PB to remove non-specifically bound DNA. A high ionic strength buffer (~200 mM) containing divalent Mg^{2+} ions was used during probe DNA grafting, where the negative charge on DNA should be screened to minimize electrostatic repulsion between DNA molecules and enhance grafting and hybridization efficiencies. DNA functionalized electrodes were incubated in 150 µl of 20 µM methylene blue (MB) prepared in 1X phosphate buffered saline (PBS) for control measurements and in 1X PBS containing either 2 mg/ml BSA or 10% fetal bovine serum (FBS) for complex media experiments for 10 minutes. 1X PBS (obtained from Corning) has a composition of 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 1.8 KH_2PO_4 with a pH of 7.4. BSA (96%) and FBS (heat inactivated) were obtained from Sigma Aldrich and Life technologies respectively. The electrodes were washed with PB after MB accumulation to remove unbound MB molecules. Subsequently, the electrode was placed inside a custom-built Teflon electrochemical cell and 1X PBS or 1X PBS containing either 2 mg/ml BSA or 10% FBS as the test case was used for subsequent electrochemical measurements. Probe-modified electrode was challenged with different concentrations of target DNA. The electrode was incubated with desired target DNA prepared in PB containing 50 mM $MgCl_2$ (control measurements) and with the addition of BSA or in 10% FBS (complex media experiments) for 35 minutes at 37 °C. The electrode was then rinsed to remove non-specifically bound target molecules. MB accumulation was again performed in a similar manner.

All electrochemical measurements were performed in a homemade Teflon cell interfaced with a Gamry Reference 600 potentiostat. Working electrodes (pl-Au and np-Au) had a footprint of 0.15 cm^2. Platinum wire and Ag/AgCl electrodes were used as counter and reference electrodes, respectively. Square wave voltammetry (SWV) was carried out for probe only electrode and then after each target hybridization in electrolyte (1X PBS for control experiments and in 1X PBS containing either 2mg/ml BSA or 10% FBS as the case may be for complex media experiments over the potential range of 0 to –0.5 mV with an amplitude of 40 mV, step size of 4 mV, and pulse frequencies ranging from 3 Hz to 60 Hz.

DNA detection mechanism

Scheme 1 includes a graphical illustration of the detection mechanism. The np-Au and pl-Au electrodes were functionalized with DNA probes and their response to MB was interrogated via square wave voltammetry (SWV). SWV measurements were performed in corresponding electrolyte after MB accumulation. A decrease in peak current amplitude was observed with successive SWVs on the probe immobilized electrodes. The peak current from the first SWV was considered as the probe baseline. The electrodes were then challenged with different target DNA concentrations. The electrodes were further incubated with MB to quantify the amount of target DNA hybridized. A decrease in SWV amplitude was observed upon target hybridization compared to the probe baseline as shown in Figure S4, which was attributed to the hindrance of MB interaction due to lack of free guanine bases in dsDNA. The difference in peak current between the probe baseline and target was used to quantify the hybridization efficiency in each case.

Schematic illustration of sensor preparation and detection mechanism



Scheme S1: DNA sensor preparation steps from cleaning the surface to functionalization with probe and target DNA, redox marker (MB) accumulation and detection are illustrated.

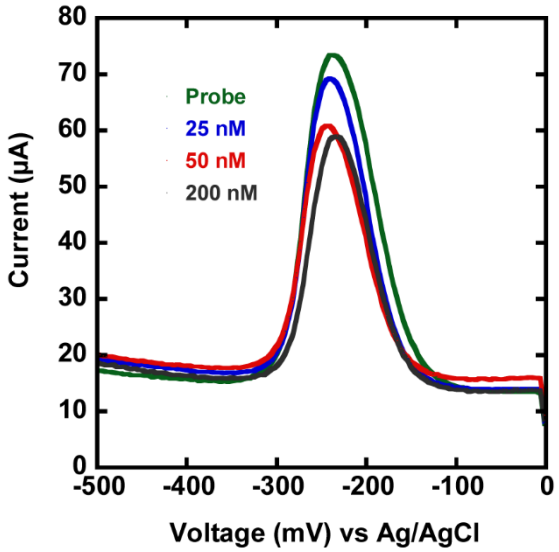


Figure S4: Square wave voltammograms (SWV) of probe and target DNA (increasing concentration) on unannealed np-Au electrodes in presence of BSA. Electrolyte: 1X PBS containing 2 mg/ml BSA at pH: 7.4

Statistical analysis

Each set of experimental conditions was verified with at least three different electrodes (np-Au and pl-Au). The reported values represent averages of three independent measurements and standard error bars. Student's t-test was used to identify differences between sample groups with different experimental conditions and p-values less than 0.05 were deemed statistically significant.