Supplementary information

Gold-sputtered microelectrodes with built-in gold reference and counter electrodes for electrochemical DNA detection

Saranya Thiruvottriyur Shanmugam [a, b], Stanislav Trashin [a, b], Karolien De Wael [a, b*]

[a] *AXES Research Group, Department of Bioengineering, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerp, Belgium.*

[b] *NANOlab Center of Excellence, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerp, Belgium*

Contents:

1. Procedure for polishing and electrochemical pretreatment of gold disk electrodes

Gold disk electrodes (BASi®, 1.6 mm in diameter) were polished for 2 min with 1 µm alumina (Buehler) slurry in water followed by 1 µm and 0.25 µm diamond slurry in alcohol lubricant (DP-Spray P, Struers) and, finally, 0.05 µm alumina (Buehler) slurry in water. After each polishing step, the electrodes were sonicated in 70% ethanol (after diamond slurry) and ultrapure water (after alumina slurry) to remove the residues of the polishing materials from the electrodes. Afterwards, the electrodes were subjected to a cyclic potential sweep for 50 cycles at 2 V/s between −0.35 and −1.4 V versus SCE (saturated calomel electrode, from Radiometer Analytical SAS). This was followed by a cyclic potential sweep in 0.5 M H_2SO_4 from 0.25 V to 1.5 V versus SCE at 0.1 V/s until a characteristic voltammogram [1, 2] was obtained.

2. Scanning electron microscopy of electrodes with and without electrochemical pretreatment

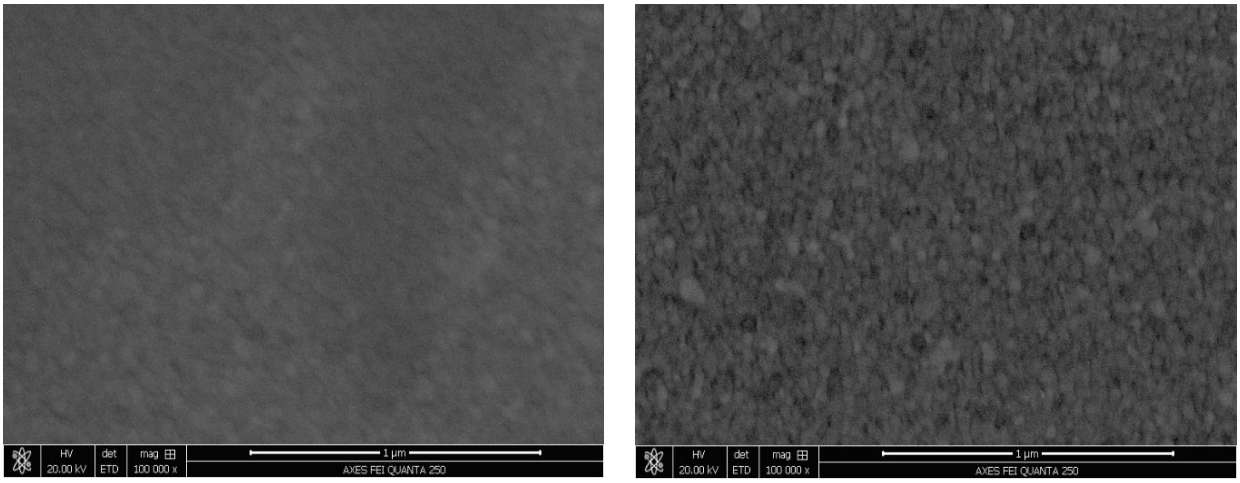

Figure S1. Scanning electron microscopy (SEM) images of the gold-sputtered electrodes after cleaning in an ultrasonic bath with 70% ethanol and water (Protocol 2) and after additional electrochemical pretreatment (Protocol 5).

3. Evaluation of desorption potential for potential pulse assisted deposition

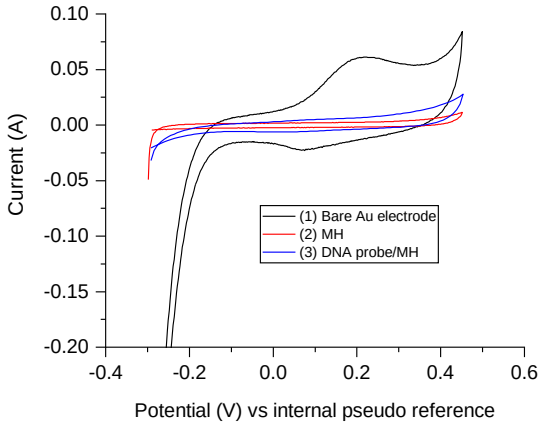

Figure S2. Evaluation of the potential window of the electrodes in the immobilization buffer used for the potential pulse assisted immobilization: (1) a bare gold-sputtered electrode, (2) an electrode coated with 1 mM

mercaptohexanol; (3) an electrode coated with 2 µM of ssDNA probe and mercaptohexanol according to the immobilization procedure. Scan rate, 0.1 V/s ; 450 mM K_2SO_4 containing 10 mM KH_2PO_4 pH 7.0.

4. Regeneration of Electrodes

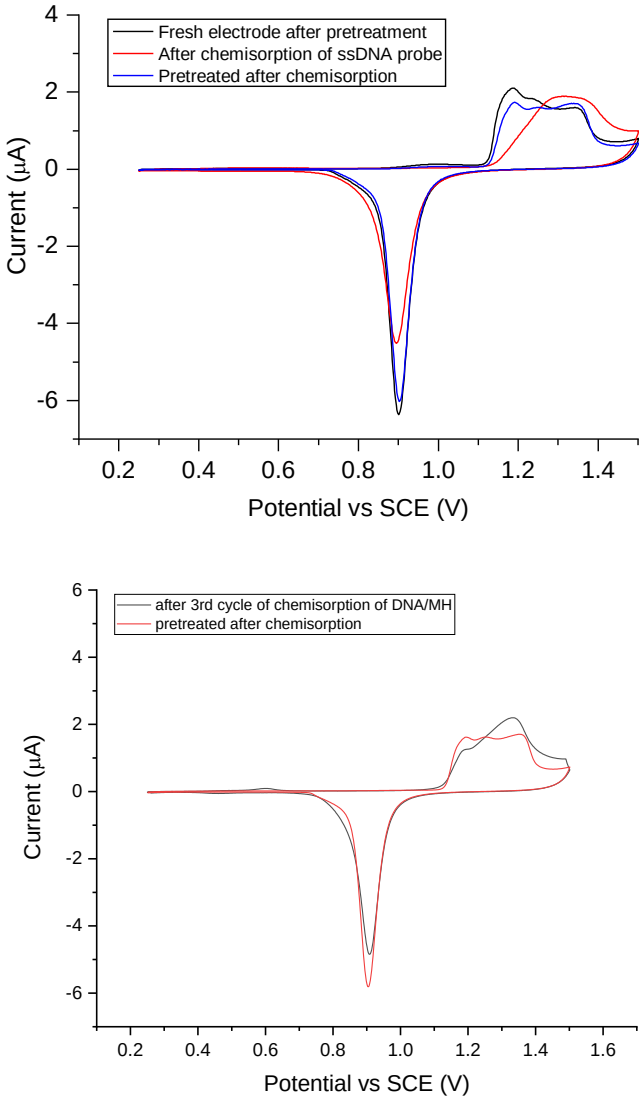

Figure S3. Top: Voltammetric behaviour of gold-sputtered electrodes in 0.5 M H_2SO_4 (Test-CV) for (1) a new freshly cleaned and pretreated electrode according to Protocol 5; (2) an electrode modified by overnight chemisorption of MH/ssDNA according to the immobilization procedure; (3) renewed by repeating the pretreatment according to Protocol 5. Bottom: Voltammetric behaviour of gold-sputtered electrodes in 0.5 M H_2SO_4 (Test-CV) for an electrode after three cycles of MH/ssDNA immobilization and detection; renewed by repeating the pretreatment according to protocol 5. Scan rate, 100 mV/s; the second scan is shown. One can notice suppression of the first peak of the gold oxide formation at around 1.16 V and decrease of the peak area at the back reduction of the gold oxide layer after introducing the organic molecules to the gold surface. The distinct profile of the gold oxide formation and the peak area for the gold oxide reduction is fully recovered after the pretreatment according to Protocol 5. The electrodes were not reusable after 3 cycles of modification and detection due to drift in the reference potential and colour change in the counter electrode possibly due to wear and tear of the microelectrode

5. DNA immobilization

Figure S4: Microelectrodes kept in humid conditions with wet tissue papers in the vicinity and sealed for overnight immobilization of ssDNA through chemisorption.

6. Built-in gold reference electrode- OCP vs SCE

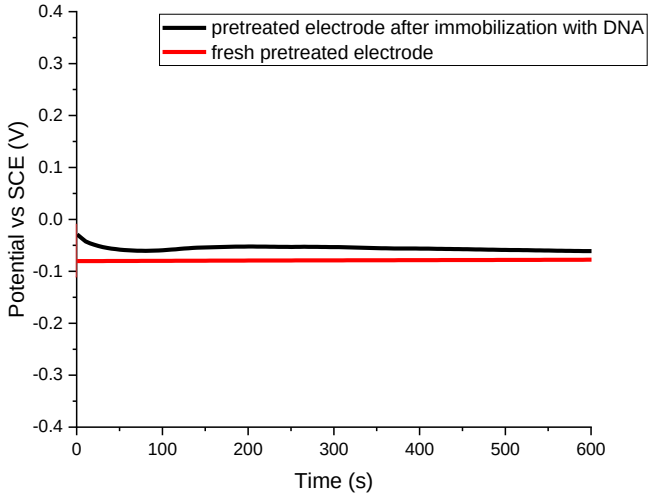

Figure S5: Open circuit potential between SCE and gold built-in reference electrode from freshly pretreated (protocol 5) bare microelectrode and pretreated (protocol 5) microelectrode after DNA/MH modification.

7. Dimensions of the gold-sputtered microelectrodes

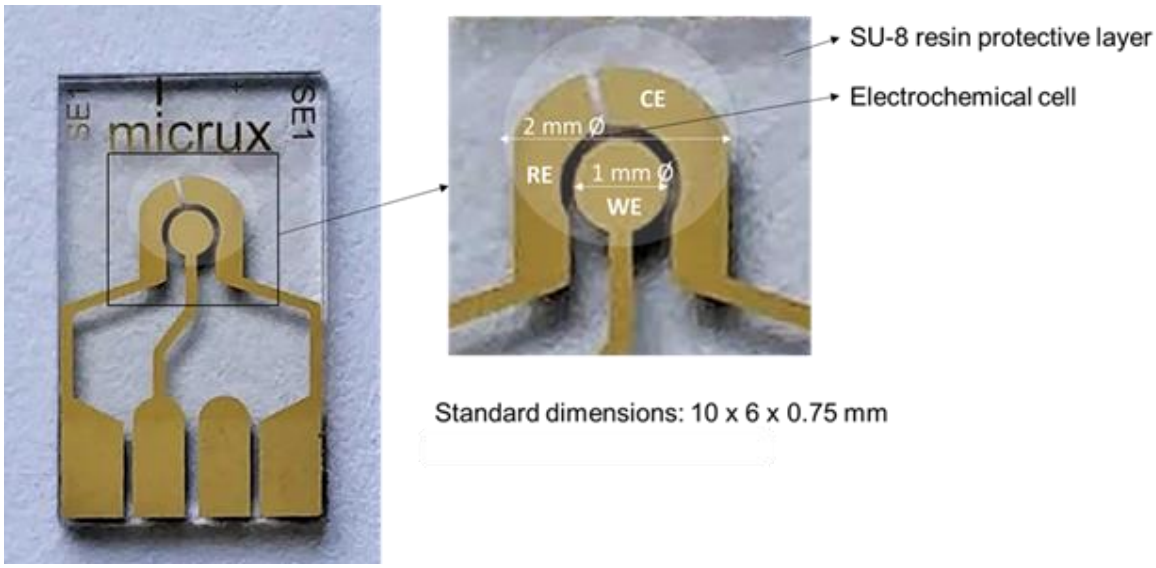

Figure S6: Photograph and dimensions of the commercially available gold sputtered microelectrode used in this study

8. Pretreatment vs target DNA detection

Table S1 Specific and unspecific responses of the sensors prepared after three different cleaning protocols.

Pretreatment protocol	Apparent R_f	Current response, I (μA)		Amplitude I_{CP}- I_{NCP}	Relative amplitude, %
		Complementary (I_{CP})	Non-complementary (I_{NCP})		
Protocol 1	1.15	0.114 ± 0.028	0.050 ± 0.012	0.064	21%
Protocol 2	1.34	0.204 ± 0.009	0.055 ± 0.007	0.148	49%
Protocol 5	1.76	0.314 ± 0.015[a]	0.013 ± 0.006	0.300	100%

[a]The response in this data set is lower compared to the response given in Figure 5 and 6 due to the use of a different batch of the detection reagents and considerably lower room temperature (18°C vs 25°C). All data in this table were obtained in the same conditions to compare three cleaning protocols.

9. Cyclic voltammetry for target DNA detection

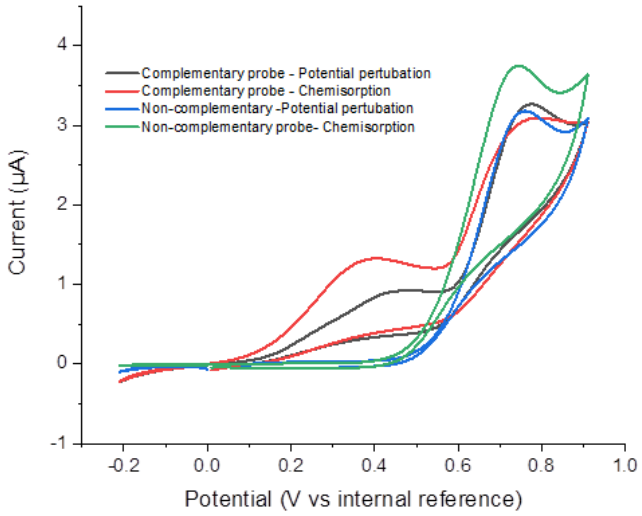

Figure S7: Cyclic voltammograms of the ssDNA modified electrodes after the DNA-detection step recorded in measuring buffer (50 mM Tris. HCl, 10 mM $MgCl_2$, pH 9.6) containing 3 mM pAPP. Scan rate, 0.1 V/s. Current vs in-built reference electrode potentials are presented here

References

1. Hoare, J.P., *A Cyclic Voltammetric Study of the Gold-Oxygen System.* Journal of The Electrochemical Society, 1984. **131**(8).
2. Burke, L.D. and P.F. Nugent, *The electrochemistry of gold: I the redox behaviour of the metal in aqueous media.* Gold Bulletin, 1997. **30**(2): p. 43-53.