## Supplementary material

## Electrochemical DNA-Biosensors based on long-range electron transfer: Investigating the efficiency of fluidic channel microelectrode compared to ultramicroelectrode in a two-electrode setup

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## 1. Impedance spectroscopy characterization.

High frequency electric impedance spectroscopy (HFEIS) measurements were performed to estimate the probe density and the ohmic resistance in the microdevice as displayed in Figures 1B-C. The instruments used are a frequency response analyser (Solartron FRA 1255B) coupled with a dielectric interface (Solartron DI 1296A). The frequency range used for EIS measurements was varied from 1 MHz to 0.1 Hz. The sinusoidal AC signal excitation between the two microelectrodes is set to 100 mV peak to peak, since DC was fixed to 0 V.

One can supposed that defects in a thiolated ss-DNA monolayer allow electroactive molecules to reach the gold electrode surface. Assuming that all the current is passed by holes on the SAM electrode, the electrode coverage can be estimated by measuring the charge transfer resistance ( $R_{ct}$ ) and by comparing the  $R_{ct}$  ratio between a bare gold surface and the surface blocked by the SS-DNA thiol immobilized.

The probe density was determined from the percentage of the coverage surface,  $\theta$ , as follows,

$$1 - \theta = \frac{R_{ct,t=0}}{R_{ct,t}} \tag{S1}$$

with,  $R_{\rm ct,t=0}$ , is the charge transfer resistance of the bare gold electrodes and,  $R_{\rm ct,t}$ , is the charge transfer resistance of the surface blocked by the SS-DNA thiol immobilized.

The charge transfer resistance, can be estimated as the difference on the real-axis between the asymptotic resistance limit at low frequency,  $R_{LF}$ , and that as frequency tends towards  $\infty$ ,  $R_{HF}$ , (Figure S1). As one can observe on real-axis,  $R_{\rm HF}$ , traditionally name the ohmic resistance,  $R_{\rm e}$ , is negligible in comparison with  $R_{\rm LF}$ .

$$R_{ct} = R_{LF} - R_e \tag{S2}$$

From data displayed on Figure S1, the ohmic resistance is estimated around 4.1 k $\Omega$ , the charge transfer resistance for the bare Au electrode and for the thiolated ss-DNA electrode over 2 hours are found equal to  $R_{\rm ct,t=0}$ = 210 k $\Omega$  and  $R_{\rm ct,t=2h}$ = 1.1 M $\Omega$ , respectively.

According to equation (S1), a total coverage of 80% of the electrodes with ss-DNA probes was obtained. By taking into account that the surface of the WE and the CE gold electrodes is  $S_{tot} = 6.09 \times 10^{-3} \text{ cm}^2$ , we deduce that the surface covered by ss-DNA is  $A_{ss-DNA} = 4.9 \times 10^{-3} \text{ cm}^2$ 

Moreover, if we assume that the DNA strands are in upright position,<sup>1</sup> the DNA head surface,  $A_{head}$ , can be calculated as follows,

$$A_{head} = \pi d_{head}^2$$
(s3)

With,  $d_{\text{head}}$ , the diameter of a DNA head estimated around 2 nm.

As a consequence, the number of DNA probes,  $N_{\text{SS-DNA}}$ , immobilized is estimated around  $3.92 \times 10^{10}$  molecules and thus the binding site density (6.44×10<sup>12</sup> sites cm<sup>-2</sup>) can be calculated as follows,

$$b_m = \frac{N_{ss-DNA}}{A_{tot}} \tag{54}$$

Similarly,  $\Gamma_{p}$ , the surfacic concentration (1.07×10<sup>-11</sup> mol cm<sup>-2</sup> given in sections 2.4 and 3.2) is given by  $b_m/Na$ , with Na being the Avogadro's number.



Figure S1. A. Nyquist representation of HFEIS data between the two microelectrodes as displayed in Figure 1B-C filled with 3 mM equimolar concentration  $[Fe(II)(CN)_6]^{3^-}$  /  $[Fe(II)(CN)_6]^{4^-}$  in 0.5 M NaCl. The applied potentials are  $V_{DC} = 0$  V and  $V_{AC} = 100$  mV for frequencies from 1 MHz to 0.1 Hz. The diameter loop allows a quick estimation of ohmic resistance, Re  $\approx 4.1$  k $\Omega$ , and of the charge transfer resistances,  $R_{ct} \approx 210$  k $\Omega$  and 1.1 M $\Omega$  for the bare gold and the thiolated ss-DNA electrode (after 2h according to the protocol described in 2.4 section), respectively.

[1] KELLEY, Shana O., BARTON, Jacqueline K., JACKSON, Nicole M., et al.Orienting DNA helices on gold using applied electric fields. Langmuir, 1998, vol. 14, no 24, p. 6781-6784.