# Nanostructuring of Sensors Determines the Efficiency of Biomolecular Capture

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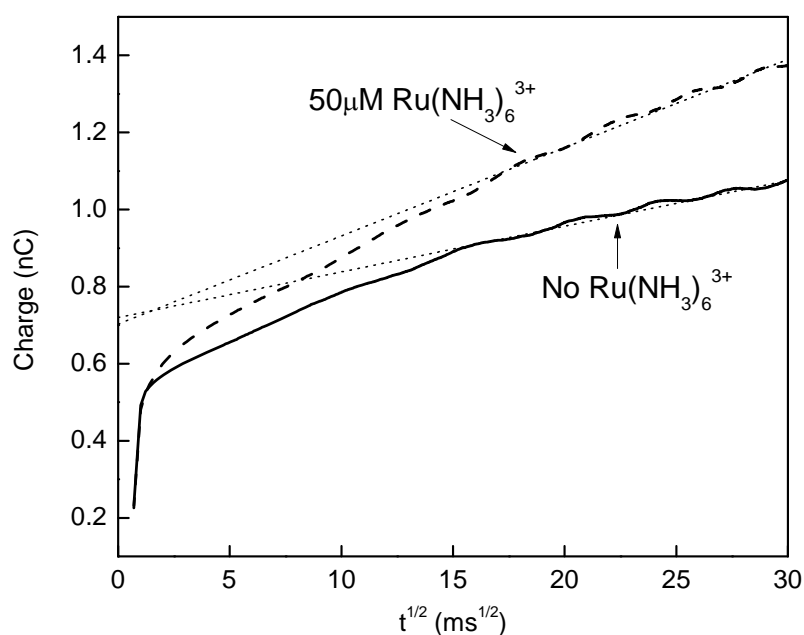
**Figure S1.** Chronocoulometric measurements of pure MCH modified NME surface in the absence and presence of 50μM $Ru(NH_3)_6^{3+}$. (……) is the least sqaures fit to linear region.

**1. Preparation and purification of oligonucleotides**

A 20-mer single stranded probe DNA oligonucleotides modified on the 5′-terminus with a hexanediamine-based linker were prepared and purified as described previously.[1] The probe (DP32) and its complementary target (T32) have the following sequences: DP32: SH- 5'- ATC TGC TCT GTG GTG TAG TT -3' and T32: 5'- AAC TAC ACC ACA GAG CAG AT -3'. All oligonucleotides were stringently purified using reversed-phase HPLC. Oligonucleotides were quantitated by measuring 260 nm absorbance using extinction coefficients calculated from the Integrated DNA technologies Web site (http://www.idtdna.com/analyzer/Applications/OligoAnalyzer/).

2. **Chip fabrication**[2]

Chips were fabricated at the Canadian Photonics Fabrication Center as described. 6" silicon wafers were passivated using a thick layer of thermally grown silicon dioxide. A 300 nm gold layer was deposited on the chip using electron-beam assisted gold evaporation. Gold film was patterned using standard photolithography and a lift-off process. A 500 nm layer of insulating silicon dioxide was deposited using chemical vapor deposition. 5 μm apertures were imprinted on the electrodes using standard photolithography. In addition, 2 mm x 2 mm bond pads were exposed using standard photolithography.

3. **Fabrication of the nanostructured microelectrodes (NMEs)**[2]

Chips were cleaned by rinsing in acetone, IPA, and DI water for 30 s and dried with a flow of nitrogen. All electrodeposition was performed at room temperature with a Bioanalytical Systems Epsilon potentiostat with a three-electrode system featuring an Ag/AgCl reference electrode and a platinum wire auxiliary electrode. 5μm apertures on the fabricated electrodes

were used as the working electrode and were contacted using the exposed bond pads. The smooth Pd NMEs were fabricated in a Palladium bath containing 5 mM solution of $H_2PdCl_4$ and 0.5 M HCl at 0 mV for 450 s using DC potential amperometry. The moderately nanostructured Pd NMEs were fabricated with the same solution as smooth Pd NMEs but with a deposition potential of -100mV for 300 s. The Pd NMEs with the finest level of nanostructuring were fabricated by two steps. First a small smooth Pd NMEs were generated (300 seconds at 0 mV) as a base, and then on the top of them the finest nanostructure was generated in a palladium bath containing 5 mM solution of $H_2PdCl_4$ and 0.5 M $HClO_4$ at -250 mV for 10 s using DC potential amperometry.

4. **Surface area determination**

The surface area of Pd NMEs was evaluated by integrating the Pd oxide reduction peak area recorded using cyclic voltammagram in the presence of 0.05M $H_2SO_4$. The electrodes were cycled in sulfuric acid by scanning the potential between the oxidation and reduction of palladium, 0.1V and 1.20V versus an Ag/AgCl reference electrode. In each cycle, a monolayer of chemisorbed oxygen is formed and reduced. The reduction charge per microscopic unit area has been experimentally determined as $424\mu C/cm^2$.[3] The microscopic surface area was obtained by integrating the reduction current peak (0.39 V vs. Ag/AgCl) to obtain the reduction charge, and dividing this by as $424\mu C/cm^2$.

5. **Modification of NMEs with ssDNA probe and hybridization protocol.**

Single-stranded thiolated 20-mer DNA probes (ssDNA DP32) were immobilized on Pd NMEs in solution containing 5 μM SH-DNA, 25 mM sodium phosphate (pH 7), 25 mM NaCl, and 50 mM $MgCl_2$ in a dark humidity chamber at room temperature for 1 hour. The NMEs were

then exposed to a 100µM mercaptohexanol (MCH) solution at room temperature for 1 hour again, to replace non-specific interaction between the DNA and palladium and form a self-assembled monolayer (SAM) that resists non-specific adsorption of target DNA. Between each step, the NMEs were rinsed in a buffer containing 25 mM sodium phosphate (PH 7), and 25mM NaCl (25/25). After carefully washing with 25/25 buffer, ssDNA modified Pd NMEs were incubated in the buffer solution (25/25) containing 1 nM target DNA and 50 mM $Mg^{2+}$ at 37°C for 1 hour.

## 6. Surface coverage and hybridization efficiency

The ssDNA surface coverage was determined using a chronocoulometric method based on that reported by Steel *et al.*[4] The DNA functionalized NMEs were first immersed in 25/25 pure electrolyte buffer, the potential stepped from + 0.15V to −0.45V versus Ag/AgCl for 100 *ms* and the resulting charge flow measured. The electrode was then immersed in a solution of 50µM hexaammineruthenium(III) chloride ($Ru(NH_3)_6^{3+}$) in 25/25 buffer, and the measurement repeated. Both solutions were purged with argon for at least 20 min prior to the experiment. In the low ionic strength buffer, the trivalent $Ru(NH_3)_6^{3+}$ preferentially exchanges with the native monovalent DNA counterions until they are essentially completely replaced, electrostatically associating to the singly negatively charged DNA phosphate groups in the ratio 1:3. At +0.15V insignificant reduction takes place, whereas −0.45V is sufficient to reduce all surface confined $Ru(NH_3)_6^{3+}$ and enforce a diffusion-limited current. The charge $Q$ as a function of time $t$ from the potential step is the sum of the reduction of $Ru(NH_3)_6^{3+}$ diffusing from solution, the double layer charge and the charge due to reduction of surface confined $Ru(NH_3)_6^{3+}$ and is given by the integrated Cottrell equation:

$$Q = \frac{2nFAD_0^{\frac{1}{2}}C_0^*}{\pi^{\frac{1}{2}}}t^{\frac{1}{2}} + Q_{dl} + nFA\Gamma_0 \qquad (1)$$

where $n$ is the number of electrons per molecule for reduction, $F$ the Faraday constant (C/mol), $A$ the electrode area (cm$^2$), $D_0$ the diffusion coefficient (cm$^2$/s), $C_0^*$ the bulk concentration of $Ru(NH_3)_6^{3+}$ (mol/cm$^3$), $Q_{dl}$ the capacitive charge ($C$) and $nFA\Gamma_0$ the charge from the reduction of $\Gamma_0$, the amount of surface confined redox marker (mol/cm$^2$). Chronocoulometric data is plotted as an Anson plot of $Q$ versus $t^{1/2}$. Extrapolation of a least squares fit to the linear part was used to determine the intercept at time zero, which corresponds to $Q_{dl} + nFA\Gamma_0$. Assuming the double layer capacitance to be approximately equal in measurements with and without $Ru(NH_3)_6^{3+}$, $Q_{dl}$ for the fixed voltage step is constant and $nFA\Gamma_0$ is calculated as the difference in intercepts.

The DNA surface coverage is determined from the surface excess of $Ru(NH_3)_6^{3+}$ as:

$$\Gamma_{DNA} = \Gamma_0\left(\frac{z}{m}\right)N_A \qquad (2)$$

where $\Gamma_{DNA}$ is the probe surface coverage (molecules/cm$^2$), $m$ is the number of phosphate groups on the probe DNA, $z$ is the charge on the redox molecule and $N_A$ is Avogadro's number.

After carefully washing with 25/25 buffer, ssDNA modified Pd NMEs were incubated in the buffer solution (25/25) containing 1 nM target DNA and 50 mM Mg$^{2+}$ at 37°C for 1 hour. Hybridization efficiency (HE) can be obtained from the following equation:

$$HE\% = \frac{\Gamma_{dsDNA} - \Gamma_{ssDNA}}{\Gamma_{ssDNA}} \times 100\% \qquad (3)$$

where $\Gamma_{ssDNA}$ and $\Gamma_{dsDNA}$ are surface coverage before and after hybridization. Care must be taken when measuring the $Q_{dl}$ after hybridization. Two factors have been considered to affect the preciseness of its value. First, partial loss of duplex in the low ionic strength of pure 25/25 buffer in the absence of $Ru(NH_3)_6^{3+}$. Secondly, the residue of $Ru(NH_3)_6^{3+}$ associated with DNA from

the last step. This will result in an inaccurate determination of the hybridization efficiency. In order to eliminate the above problems, the step for determination of $Q_{dl}$ of Pd NMEs after hybridization were omitted for this chip and directly measure $\Gamma_{dsDNA}$ in electrolyte buffer containing 50 μM $Ru(NH_3)_6^{3+}$. In addition, a second parallel chip of Pd NME samples was prepared to determine the ratio ($r$) of $Q_{dl}$ after and before hybridization separately. The values of $Q_{dl}$ of ssDNA modified NMEs on this chip were obtained in pure 25/25 buffer by chronocoulometry, this chip were then directly hybridized with target DNA solution without involving $Ru(NH_3)_6^{3+}$ containing solution, after hybridization, the $Q_{dl}$ of dsDNA was measured in pure 25/25 electrolyte again. The obtained $r$ values (1.5-1.8) were then used to calculate the $Q_{dl}$ after hybridization of the first chip, by multiplying its $Q_{dl}$ before hybridization (ssDNA) by $r$.

**7. Discussion of $Q_{dl}$ correction.** In experiments monitoring $Q_{dl}$ changes before and after hybridization, it was observed that this parameter did vary as a function of hybridization state. Careful measurements were therefore done to assess this as a function of working surface area so that a precise correction could be made for each sensor. Representative data is shown below.

Table S1. $Q_{dl}$ values of ssDNA and dsDNA of three different Pd NMEs

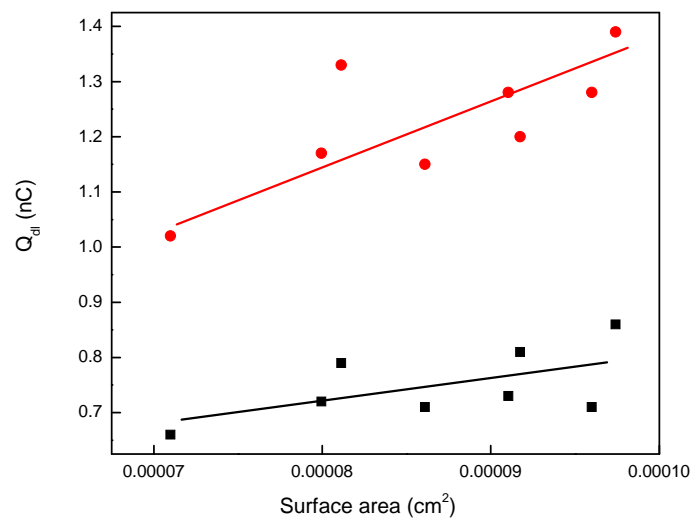| NMEs | ssDNA ($μC/cm^2$) | dsDNA ($μC/cm^2$) |
|---|---|---|
| Smooth | $7.9 \pm 0.9$ | $11.5 \pm 1.9$ |
| Moderately nanostructured | $8.7 \pm 0.7$ | $14.2 \pm 1.1$ |
| Finely nanostructured | $14.6 \pm 2.1$ | $21.9 \pm 1.6$ |

Figure S2. Measurement of $Q_{dl}$ for sensors with varied working areas as measured by oxide stripping

## References

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