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Electronic transduction of biocatalytic transformations on nucleic acid-functionalized surfaces†

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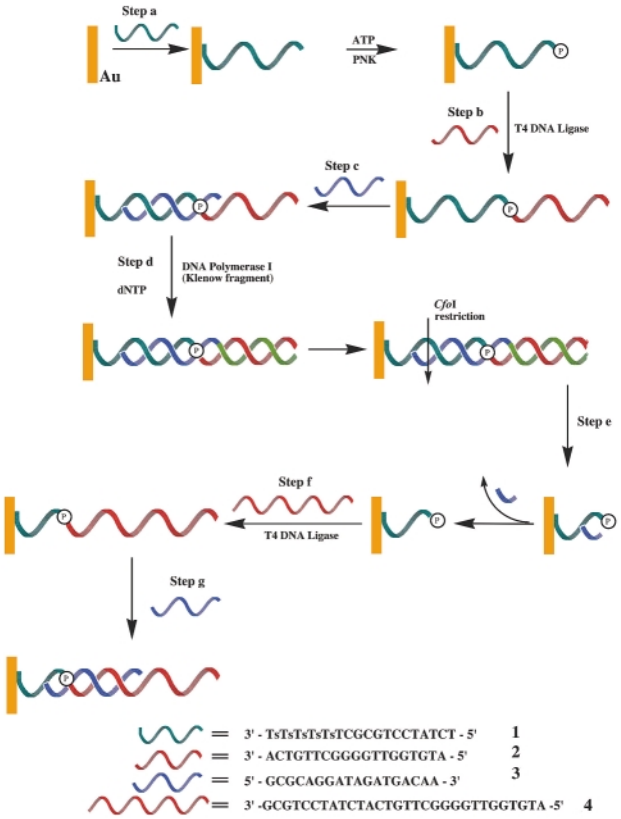
Electronic transduction of enzyme-catalyzed transformations on nucleic acids associated with surfaces such as ligation, polymerization or restriction, is accomplished.

The use of nucleic acid interactions to construct organized nanostructures,[1,2] circuits[3] and nanomachines,[4] the use of the encoded information in nucleic acids for the assembly of DNA-based computers,[5,6] and the electronic transduction of DNA detection processes[7,8] represent major research efforts in the rapidly developing area of DNA bioelectronics. The hybridization of nucleic acids, and the biocatalyzed ligation, replication and scission of DNA are basic tools to detect nucleic acid interactions and to generate DNA structures. Recently, Faradaic impedance spectroscopy[9] and microgravimetric quartz-crystal-microbalance (QCM) measurements[10] were used as electronic transduction means for the nucleic acid recognition events on conductive supports. Here we report on the novel electronic transduction of a series of biocatalyzed transformations involving nucleic acids that include the surface-stimulated ligation, replication and the specific scission of nucleic acids by a restriction enzyme.

The 18-mer oligonucleotide, **1**, was assembled on an Au-electrode or on an Au/quartz piezoelectric crystal (9 MHz, AT-cut) by the association of the thymine thiophosphate-tag to the gold supports.[11] The surface coverage of the oligonucleotide **1** was estimated by QCM to be $(5.0 \pm 0.7) \times 10^{-11}$ mol cm^{-2}. The resulting **1**-functionalized surfaces were reacted with polynucleotide kinase, PNK, in the presence of ATP to phosphorylate the 5′ termini of the oligonucleotide-monolayer.† The resulting interface was reacted with **2** in the presence of ligase,† Scheme 1, to induce the ligation of **2** to the base oligonucleotide associated with the surface. Fig. 1(A) shows the Faradaic impedance spectra observed upon performing the biocatalyzed transformations on the nucleic acids associated with the electrode, whereas Fig. 1(B) shows the respective frequency changes occurring on the piezoelectric crystal as a result of the chemical transformations occurring on the crystal.† The ligation of **2** results in an increase in the interfacial electron transfer resistance from $R_{et} = 0.44$ kΩ to $R_{et} = 1.33$ kΩ (Fig. 1(A), curve (b)). This is consistent with the fact that the increase of the negative charge associated with the electrode, as a result of ligation, enhances the electrostatic repulsion of the redox-label, $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$, thus increasing the interfacial electron transfer resistance. The frequency of the Au/quartz crystal changes upon ligation by $\Delta f = -100$ Hz, a value that translates to a surface coverage of the ligated product corresponding to $(5.1 \pm 0.8) \times 10^{-11}$ mol cm^{-2}.† No frequency changes of the Au/quartz crystal were observed upon an attempt to ligate **2** to the **1**-functionalized crystal without the primary phosphorylation of the interface by PNK. Also no frequency changes of the crystal were observed upon interacting the phosphorylated functionalized crystal with **2** in the absence of ligase. The resulting nucleic acid associated with the interface was hybridized with **3**, that is complementary to a part of the nucleic acid associated with the solid supports. The interfacial electron transfer resistance increases as a result of the hybridization of **3**, $R_{et} = 1.9$ kΩ, Fig. 1(A), curve (c), consistent with the increase of the negative charge associated with the electrode. The frequency of the quartz crystal changes by $\Delta f = -31$ Hz, that corresponds to a surface coverage of the hybridized assembly of $(2.2 \pm 0.4) \times 10^{-11}$ mol cm^{-2}. The incomplete hybridization is attributed to steric constraints on the electrode support that eliminate the formation of dsDNA with all of the nucleic acid components.

The resulting assembly was then reacted with the mixture of nucleotides, dNTP, in the presence of polymerase (Klenow fragment, DNA polymerase I).† This yields an increase in the interfacial electron transfer resistance, $R_{et} = 3.1$ kΩ, as a result of the higher negative charge associated with the interface, Fig. 1(A), curve (d). The change in the frequency of the piezoelectric crystal as a result of polymerization is $\Delta f = -26$ Hz, indicates a surface coverage of $(2.6 \pm 0.4) \times 10^{-11}$ mol cm^{-2} for the replicated product.† Reaction of the assembly with the endonuclease restriction enzyme *Dra*I that stimulates the



Scheme 1 Biocatalyzed ligation, replication and scission of single and double stranded DNA on electronic transducers (Ts = thymine thiophosphate).

† Electronic supplementary information (ESI) available: details of the experimental conditions for the biocatalytic transformations on the electrodes and a histogram of the frequency changes observed in a series of different experiments. See http://www.rsc.org/suppdata/cc/b1/b104335h/

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specific scission of 5′TTT/AAA3′ sequence does not yield any change in the impedance spectrum of the assembly. Reaction of the resulting assembly with the endonuclease restriction enzyme *Cfo*I (*Hha*I) that induces the specific scission of the 5′GCG/C3′ sequence results, however, in the cleavage of the ds-assembly,† *cf.* Scheme 1. The resulting Faradaic impedance spectrum is shown in Fig. 1(A), curve (e). The interfacial electron transfer resistance decreases to R_e = 0.9 kΩ. This is consistent with the fact that removal of a major part of the ds-assembly and the negative charge associated with it, by the endonuclease activity, reduces the barrier for electron transfer between the redox-label and the electrode. The frequency change in the Au/quartz crystal as a result of the endonuclease activity is Δf = +75 Hz, implying a decrease in the mass associated with the crystal, Fig. 1(B). From the frequency change we estimate that *ca.* $(1.5 \pm 0.2) \times 10^{-11}$ mol cm^{-2} of the hybridized nucleic acid underwent scission. The scission of the double-stranded DNA yields a 5′-phosphorylated primer on the electrode. Note that the interfacial electron transfer resistance of the resulting electrode is higher than the electron transfer resistance of the **1**-functionalized electrode despite the fact that the endonuclease cleavage generates a shorter oligonucleotide than **1** on the electrode. This is explained by the fact that the *Cfo*I cleavage proceeds with partial efficiency, thus leaving a substantial surface coverage of the ds-replicated DNA on the electrode support (*ca.* 1.1×10^{-11} mol cm^{-2}). The resulting interface was then reacted with the oligonucleotide **4** in the presence of ligase to yield the original surface, Fig. 1(A), curve (f), exhibiting an electron transfer resistance of R_{et} = 2.2 kΩ. Further hybridization of **3** with the ligated interface results in an additional increase in the electron transfer resistance to R_{et} = 2.6 kΩ, Fig. 1(A), curve (g).‡ The ligation of **4** to the interface, and the hybridization of **3** with the interface yield, however, higher interfacial electron transfer resistances than those observed for the originally functionalized electrodes, curves (b) and (c), respectively. This is consistent with the fact that endonuclease-induced scission proceeds with partial efficiency, and thus the secondary ligation and hybridization occurs on an interface that includes a partial coverage of the polymerase-induced replicated double-stranded DNA. The negative charge associated with these latter components introduces the higher interfacial electron transfer resistances observed in the second cycle of the biocatalytic transformations. The QCM analyses, Fig. 1(B) confirm this explanation. Ligation of **4** to the interface results in a frequency change of $\Delta f = -42$ Hz, corresponding to a surface coverage of *ca.* $(1.4 \pm 0.3) \times 10^{-11}$ mol cm^{-2} of the coupled product. It should be noted that no significant changes in the interfacial electron transfer resistances, upon the performance of the set of biocatalyzed transformations outlined in Scheme 1, are observed in the presence of the neutral ferrocene methanol redox-label. This supports the conclusion that the changes in the impedance spectra originate from the electrostatic repulsions of the redox-label $Fe(CN)_6^{3-/4-}$. Also, it should be noted that the Faradaic impedance spectra results reveal an excellent reproducibility (±5%) whereas the QCM results reveal a larger experimental diversity due to a different roughness of the Au/quartz crystals.

In conclusion, the electronic transduction of different biocatalytic transformations that include nucleic acids on surfaces was accomplished. This enables the quantitative assay of DNA building blocks on surfaces, and the characterization of nanoengineered DNA structures on surfaces.

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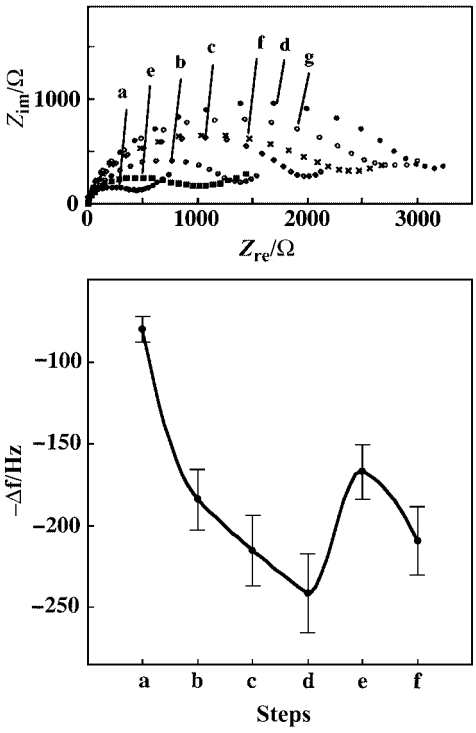

Fig. 1 (A) Faradaic impedance spectra corresponding to the biocatalytic transformation of nucleic acid-functionalized electrode: (a) **1**-functionalized electrode; (b) after ligation of **2**, 3×10^{-5} M, with the **1**-functionalized electrode in the presence of ligase, 20 units, 37 °C, 30 min; (c) after hybridization of the resulting electrode with **3**, 2.5×10^{-5} M, 2 h; (d) after replication of the double-stranded assembly in the presence of dNTP, 1×10^{-3} M, and DNA polymerase, 3 units, 37 °C, 30 min; (e) after scission of the resulting assembly with endonuclease, *Cfo*I, 10 units, 37 °C 1 h; (f) after ligation of the resulting interface with **4**, 6.5×10^{-5} M, in the presence of ligase, 20 units, 37 °C, 30 min; (g) after hybridization of the assembly with **3**, 1×10^{-4} M, for 2 h. (B) Frequency changes of an Au/quartz crystal (9 MHz, AT-cut) upon the assembly of the **1**-functionalized interface and as a result of the biocatalyzed transformations/processes occurring on the surface. Steps (a)–(f) correspond to the steps and preparation protocols outlined in (A).

Notes and references

‡ The different Faradaic impedance spectra of the DNA assemblies on the electrodes can be theoretically fitted with an equivalent circuit composed of a block of an electron transfer resistor element that is linked in series to the Warburg element in parallel to a capacitor element.

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