2962, 2246, 1708, 1647, 1537, 1500, 1249, 1024, 753 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, 60 °C): $\delta = 9.86$ (s, 1H), 7.45 (td, $J = 7.6$, 1.6 Hz, 1H), 7.33 (d, $J = 4.4$ Hz, 4H), 7.29 (m, 1H), 7.22 (m, 6H), 7.15 (m, 4H), 7.04 (m, 2H), 6.97 (t, $J = 7.4$ Hz, 1H), 6.87 (d, $J = 8.4$ Hz, 2H), 6.44 (d, $J = 8.0$ Hz, 1H), 6.11 (d, $J = 7.2$ Hz, 1H), 5.80 (s, 1H), 5.25 (d, $J = 8.8$ Hz, 1H), 5.21 (d, $J = 12.0$, 1H), 5.14 (d, $J = 12.0$ Hz, 1H), 5.08 (s, 2H), 4.93 (s, 2H), 4.64 (t, $J = 7.6$ Hz, 1H), 4.25 (td, $J = 10.0$, 2.4 Hz, 1H), 3.98 (t, $J = 8.0$ Hz, 1H), 3.13 (t, $J = 12.4$ Hz, 1H), 2.61 (d, $J = 11.2$ Hz, 1H), 2.08 (m, 2H), 1.04 (d, $J = 6.8$ Hz, 3H), 0.94 (m, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 195.8$, 172.1, 171.2, 164.6, 158.7, 157.2, 156.5, 154.3, 136.4, 136.0, 135.9, 132.5, 131.5, 130.8, 130.4, 129.3, 128.7, 128.6, 128.5, 128.3, 128.2, 128.1, 128.0, 127.9, 127.4, 127.2, 122.5, 121.7, 113.6, 113.4, 112.9, 111.7, 71.0, 67.3, 62.4, 60.3, 56.3, 55.3, 53.7, 51.0, 37.9, 31.7, 30.0, 19.6, 19.3, 18.1; MS (Positive electrospray) for $C_{52}H_{52}N_5O_8$ $[M+H]^+$: calcd: 874.4, found: 874.4; HRMS (FAB) for $C_{52}H_{52}N_5O_8$ $[M+H]^+$: calcd: 874.3815, found: 874.3797.

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Electrochemical Transduction of Liposome-Amplified DNA Sensing**

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Dedicated to Professor Heinz Dürr on the occasion of his 65th birthday

The development of DNA sensors has recently attracted substantial attention in connection with research efforts directed at gene analysis, the detection of genetic disorders, tissue matching, and forensic applications.[1, 2] Optical detection of DNA was accomplished by the application of fluorescence-labeled oligonucleotides,[3] or by the use of surface plasmon resonance (SPR) spectroscopy.[4] Electronic transduction of oligonucleotide – DNA recognition events, and specifically the quantitative assay of DNA, are major challenges in DNA-based bioelectronics.[5] Electrochemical DNA sensors based on the amperometric transduction of the formation of double-stranded oligonucleotide – DNA assemblies have been reported.[6] Also, electrostatic attraction or intercalation of transition metal complexes[7] or dyes[8] was used for the voltammetric probing of the production of double-stranded oligonucleotide assemblies. Microgravimetric quartz crystal microbalance (QCM) analyses were also applied to sense the formation of double-stranded oligonucleotide – DNA complexes on surfaces.[9]

Two fundamental issues that need to be addressed for the development of DNA sensors relate to the specificity and sensitivity of the sensing devices. We have reported on a general method to amplify biorecognition and sensing events by the biocatalyzed precipitation of an insoluble product on the electrode support.[10–12] Enzyme electrodes,[10] immunosensors,[11] and specifically DNA sensors[12] were developed by this amplification route. We have also described specific DNA sensing by the application of a three-component double-stranded sensing assembly consisting of a primer oligonucleotide that is complementary to the mutation domain, the analyte DNA, and a labeled oligonucleotide for assaying and amplifying of the recognition event.[12] Here we report on a novel method for the amplification of oligonucleotide – DNA biorecognition events using functionalized liposomes.[13] The DNA sensing events are transduced electrochemically, using Faradaic impedance spectroscopy. Electronic DNA sensors of unprecedented specificity and sensitivity have been organized, and they exhibit a 10^5- to 10^6-fold enhancement of sensitivity over previous electrochemical DNA sensors.[6, 14]

One DNA sensing configuration is outlined in Scheme 1 A. The sulfanylhexyl oligonucleotide **1** was assembled as a monolayer on an Au electrode. The surface coverage (1.1×10^{-11} mol cm^{-2}) was determined by the electrochemical meth-

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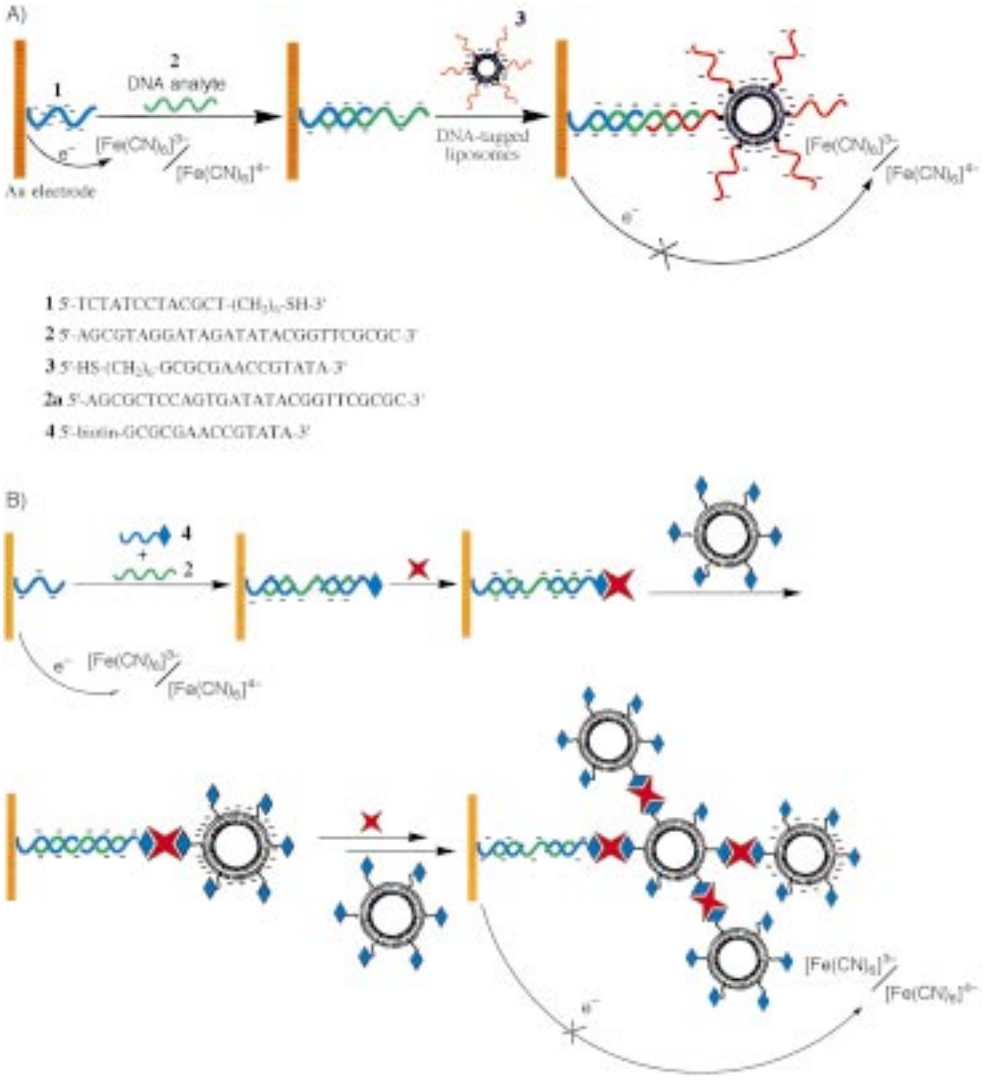

Scheme 1. A) Amplified electrochemical sensing of an analyte DNA using oligonucleotide-functionalized liposomes and Faradaic impedance spectroscopy as a means of transduction. Oligonucleotides **1**, **2**, and **3** are represented by blue, green, and red wavy lines, respectively. B) Electrochemical sensing of an analyte DNA using a biotinylated oligonucleotide, avidin, liposome labeled with biotin as an amplification conjugate and Faradaic impedance spectroscopy as a means of transduction. Green wavy lines: oligonucleotide **2**, blue wavy lines: oligonucleotide **4**, blue diamonds: biotin, red stars: avidin.

od of Tarlov et al.,[15] and comparable results were obtained by QCM analyses. The resulting monolayer-functionalized electrode was allowed to interact with the analyte DNA **2** (15 min hybridization, 25 °C) to yield the double-stranded assembly on the electrode surface. The resulting electrode interface was then treated with the liposome labeled with oligonucleotide **3** (15 min, 25 °C). Oligonucleotide **3** is complementary to the residual base sequence of the analyte **2**. Thus, a liposome-linked, three-component, double-stranded assembly—consisting of the primer **1**, the analyte **2**, and the liposome tagged with **3**—is generated on the electrode support. The **3**-labeled liposomes are negatively charged in order to eliminate nonspecific adsorption of the liposomes onto the sensing interface. The oligonucleotide-functionalized liposome was prepared by assembling liposomes composed of phosphatidic acid, phosphatidyl choline, maleimide-phosphatidylethanolamine, and cholesterol (labeled with ^{3}H-cholesterol, 45 Ci mol^{-1}) in a ratio of 79:20:1:0.1. The liposomes were modified with **3** (4 °C, 20 h) and purified by chromatography (Sephadex G-75).

The surface coverage of the liposome with **3** (50–60 oligonucleotide units per liposome) was determined by allowing the resulting liposomes to react with Oligreen (Molecular Probes) and following the fluorescence intensity of the resulting liposome suspension at $\lambda_{ex} = 480$ nm. The size of the liposomes was determined by dynamic light scattering experiments to be (220 ± 20) nm. The liposomes associated with the electrode support represent "giant" negatively charged interfaces that electrostatically repel a negatively charged redox probe solubilized in the electrolyte solution. Therefore, the biorecognition event between the primer **1** and the analyte DNA **2** is amplified by the generation of a highly charged microenvironment that repels the electroactive probe, $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$, in solution. This electrostatic repulsion of the redox probe introduces a barrier for interfacial electron transfer, and results in an interfacial electron transfer resistance that can be assayed by Faradaic impedance spectroscopy.[16]

Figure 1 A shows the impedance spectra (in the form of a Nyquist plot, Z_{im} vs. Z_{re}) of the oligonucleotide-functionalized electrode (curve a), after hybridization with the analyte DNA **2** (bulk concentration 5×10^{-6} M, curve b), and after interaction with the probing **3**-functionalized liposome (curve c). While a bare Au electrode exhibits an electron transfer resistance of 0.5 kΩ, the association of the primer **1** onto the conducting support increases the electron transfer resistance to 3 kΩ. This is attributed to the electrostatic repulsion of the redox label, $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$, which results in a barrier for the interfacial electron transfer. The formation of the double-stranded assembly with the analyte DNA increases the electron transfer resistance to $R_{et} = 4.5$ kΩ. This is consistent with the fact that the higher negative charge formed on the surface as a result of hybridization enhances the electrostatic repulsion of the electroactive species in solution. Binding of the **3**-modified liposome introduces a very high electron transfer resistance corresponding to 15 kΩ. This is attributed to the formation of a negatively charged

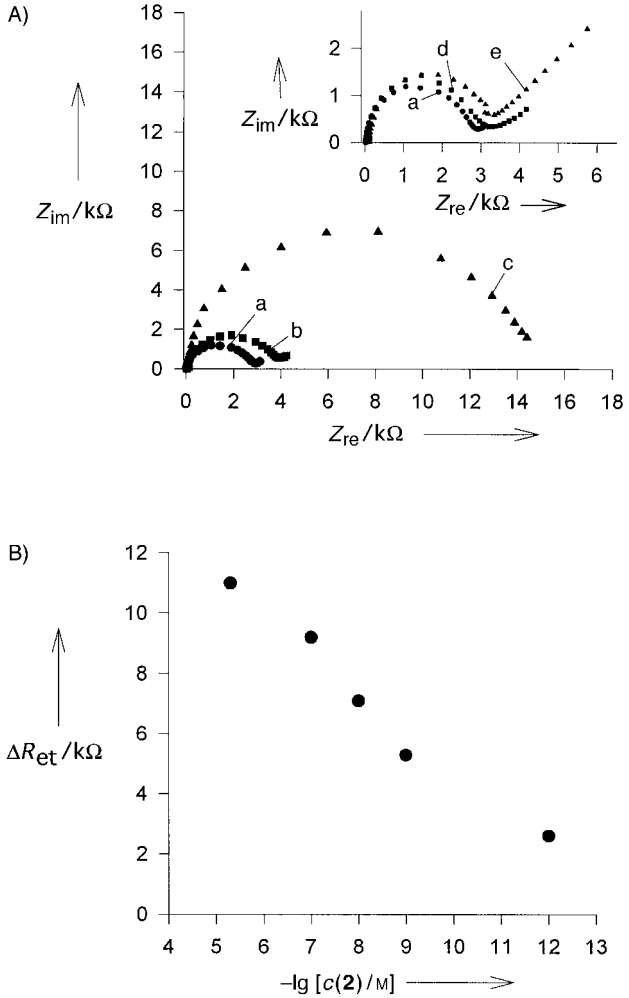

Figure 1. A) Faradaic impedance spectra (Nyquist plots, Z_{im} = imaginary impedance, Z_{re} = real impedance) of a) the **1**-functionalized Au electrode, b) after interaction of the sensing electrode with **2** (5×10^{-6} M; 15 min, 25 °C) and c) after interaction with the **3**-functionalized liposome (lipid concentration 0.2 mM, 15 min). Inset: Faradaic impedance spectra of a) the **1**-modified electrode; d) after interaction with **2a** (5×10^{-6} M); e) after treatment with **3**-functionalized liposomes (lipid concentration 0.2 mM). All measurements were performed using 0.2 cm^2 Au electrodes (roughness factor 1.2–1.5) in a 0.1 M phosphate buffer (pH 7.2) that contained $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ (5×10^{-3} M, 1:1); bias potential 0.180 V vs. SCE, frequency range 100 mHz – 10 kHz, alternate voltage 5 mV. B) Changes in the electron transfer resistance of the **1**-functionalized electrode upon treatment with the analyte DNA **2** in different concentrations and secondary amplification with the **3**-functionalized liposomes. ΔR_{et} corresponds to the difference in the electron transfer resistance after amplification with the **3**-functionalized liposome and the resistance of the **1**-modified electrode.

microinterface upon association of the liposome to the double-stranded assembly. The resulting charged interface strongly repels the redox label from the electrode interface, resulting in a high electron transfer resistance. A control experiment where only **3** binds to the double-stranded assembly of the primer oligonucleotide and the analyte DNA introduces only a small increase in the electron transfer resistance ($R_{et} = 4.7$ kΩ), indicating that the negatively charged liposome indeed amplifies the electrostatic repulsion of the redox label. A further control experiment involved an attempt to sense the DNA with **2a**, which includes a six-base mutation relative to the analyte DNA **2**.

Curve d of Figure 1 A Inset shows the impedance spectrum of the functionalized electrode after treatment with the mutant **2a**, and curve e shows the impedance spectrum of the resulting electrode after treatment with the **3**-functionalized liposome. The interfacial electron transfer resistances are almost unchanged, implying that the sensing interface is selective for the analyses of **2**. The results also indicate that no nonspecific association of **2a** or the **3**-functionalized liposomes on the electrode takes place. This is attributed to the electrostatic repulsions existing between these components and the sensing interface. The extent of increase in the electron transfer resistances upon the binding of the analyte DNA, and the secondary association of the **3**-modified liposome, is controlled by the bulk concentration of the analyte DNA (Figure 1 B). The lower sensitivity limit for analyzing the analyte DNA is 1.2×10^{-12} M (or 6×10^{-16} mol in the analyte sample). The results shown in Figure 1 were fully reproducible in a series of 25 electrodes. For the lowest sensitivity limit the value $\Delta R_{et} = (2.5 \pm 0.2)$ kΩ (signal-to-noise $(S/N) > 4$) was found.

Scheme 1 B shows a second configuration for the amplified sensing of the analyte DNA using biotin-labeled, negatively charged liposomes. The **1**-modified electrode was allowed to react with the analyte DNA **2** (15 min hybridization, 25 °C), which was pretreated with the biotinylated oligonucleotide **4** (complementary to one segment of the target DNA). This process results in a three-component double-stranded assembly on the electrode consisting of the primer **1**, the target DNA **2**, and the biotin-labeled oligonucleotide **4**. Association of avidin (8 min) and then the biotin-tagged liposome (8 min) results in the formation of a negatively charged interface that amplifies the primary DNA recognition event by the electrostatic repulsion of $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ and the enhancement of the interfacial electron transfer resistance. This sensing configuration enables further amplification of the biorecognition event by the multiple reaction of the resulting array with avidin and then with the biotinylated liposomes to yield a dense array of the negatively charged liposomes. The biotin-functionalized liposomes are composed of phosphatidyl choline, phosphatidylethanolamine, cholesterol (partially labeled with ^3H, 45 Ci mol^{-1}), and biotinylated phosphatidylethanolamine in a ratio of 80:20:0.1:0.1. The average coverage corresponds to 550 biotin units per liposome. The biotin-labeled liposomes were purified by gel chromatography (DEAE Sephadex A-25). The size of the liposomes was determined by dynamic light scattering to be (180 ± 40) nm.

Figure 2 A shows the impedance spectra of the array in the different steps of modification. The **1**-functionalized interface exhibits an electron transfer resistance of 3 kΩ (curve a). Upon the formation of the double-stranded assembly with the analyte DNA **2**, complexed to the biotinylated oligonucleotide **4**, the electron transfer resistance increases to 4.8 kΩ (curve b). Association of avidin to the interface further increases the electron transfer resistance to 7.6 kΩ as a result of the hydrophobic, insulating, features of the protein (curve c). Association of the biotin-labeled liposome to the surface substantially increases the electron transfer resistance ($R_{et} = 14.8$ kΩ, curve d). The enhanced electron transfer resistance is due to the electrostatic repulsion of the electro-

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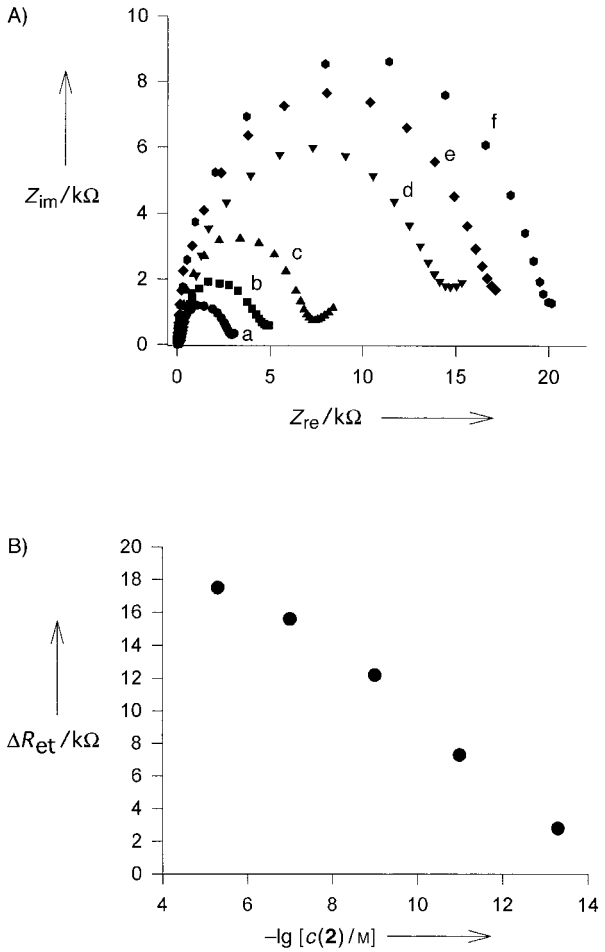

Figure 2. A) Faradaic impedance spectra (Nyquist plots) of a) the **1**-functionalized Au electrode; b) after interaction of the sensing electrode with **2** (5×10^{-6} M), which was pretreated with **4** (1×10^{-5} M, interaction time 30 min, 25 °C); c) after treatment of the resulting electrode with avidin (2.5 μg mL^{-1}); d) after interaction with the biotinylated liposomes (8 min, lipid concentration 0.25 mM); e) after treatment of the interface for a second time with avidin (2.5 μg mL^{-1}); f) after interaction of the interface for a second time with the biotinylated liposomes. All impedance experiments were performed under the conditions detailed in the legend to Figure 1 A. B) Calibration curve corresponding to the changes in the electron transfer resistance of the sensing electrode upon interaction with the analyte DNA **2** in different concentrations and enhancement of the sensing processes by a double-step avidin/biotinylated liposome amplification path. ΔR_{et} corresponds to the difference in the electron transfer resistance after a double-step avidin/biotinylated liposome amplification and the electron transfer resistance of the **1**-functionalized electrode.

active species in solution by the charged membrane interface. The sensing of the target DNA can be further amplified by the application of a second step of association of the avidin/biotinylated liposomes, which enhances the electron transfer resistances to 17 kΩ and 20 kΩ, respectively (curves e and f).

In a control experiment, the sensing interface was allowed to interact with the noncomplementary DNA **2a** (see Scheme 1 A; 5×10^{-6} M), which was pretreated with **4**, and subsequently treated with avidin and the biotinylated liposome. A minute increase in the electron transfer resistance corresponding to $R_{et} = 3.4$ kΩ was observed, which is attributed to the nonspecific adsorption of avidin to the sensing interface. The increase in the electron transfer resistance at the electrode upon binding of avidin and the biotin-labeled liposome is controlled by the bulk concentration of the analyte DNA in the sample (Figure 2 B). With a double-step pathway with amplification of the avidin/biotin-labeled liposome, analyte DNA concentrations as low as 5×10^{-14} M (or 2.5×10^{-17} mol in the analyte sample) can be determined. Although the results shown in Figure 2 represent the data for a single set of five electrodes, the data are fully reproducible, and for the lowest detectable concentration the value $\Delta R_{et} = (2.5 \pm 0.5)$ kΩ ($S/N > 3$) was observed for a series of 30 electrodes. The amplified transduction of DNA analyses by the functionalized liposomes, and with use of the two configurations, exhibits excellent reproducibility.

In conclusion, we have demonstrated novel methods to amplify oligonucleotide – DNA recognition events on electrodes using negatively charged labeled liposomes. The highly sensitive sensing processes were transduced by Faradaic impedance measurements, and the sensing interfaces revealed selectivity in the analysis of the target DNA. The possibility of organizing microelectrode arrays on transducers paves the way to develop electrochemical DNA chips for the multicomponent DNA analyses.[17]

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