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DNA hybridization "turns on" electrocatalysis at gold electrodes†

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The efficiency of electrocatalysis occurring at DNA-modified gold electrodes is highly dependently on the density of DNA monolayers, as a result, DNA hybridization can "turn on" electrocatalysis by increasing the DNA surface density.

Sequence-specific detection of either genetically or pathogenically associated nucleic acids has become increasingly important for applications including point-of-care diagnostics, antiterrorism, environmental monitoring and forensic analysis.[1,2] Therefore, it is highly desirable to develop DNA detection methods with high sensitivity and speed, which has motivated the development of various optical, electronic and acoustic DNA biosensors.[3–6] Because electrochemical detectors are inexpensive, portable and power-saving, electrochemical DNA biosensors have been widely recognized to be a highly promising approach to detect clinically, environmentally and security relevant nucleic acids, especially when time, money and/or resources are limited.[7,8]

A typical electrochemical DNA sensor involves an electrode that is modified with DNA capture probes.[7] Hybridization events of surface-confined capture probes with target DNA are coupled with redox reactions *via* various strategies, leading to electrochemical signals that manifest the existence of target DNA in test samples.[4,7–15] Apparently, detection sensitivity of electrochemical sensors depends on the ratio between total electrons flowing through electrodes and the number of hybridization events. Therefore, electrocatalysis is often employed to improve the detection sensitivity.[9–12,16,17] In such electrocatalysis-based assays, an electrode reaction is coupled with a chemical reaction that converts the electrochemically reduced or oxidized species to its original state, which forms an electrocatalytic cycle and increases the electron flux. For example, Barton and coworkers elegantly demonstrated that even single-nucleotide mismatches could be sensitively detected by coupling electroactive and intercalated methylene blue (MB) with ferricyanide ($Fe(CN)_6^{3-}$) in an electrocatalytic cycle.[9,11] More recently, Kelley and coworkers developed sensitive DNA sequence sensors by using the $[Ru(NH_3)_6]^{3+}$ (RuHex)/$Fe(CN)_6^{3-}$ electrocatalytic system.[12,18,19] While electrocatalysis has been favorably used in DNA sensors, it has rarely been exploited how DNA assembly at electrodes affects the electrocatalytic efficiency. In this communication, we systematically interrogated the effect of DNA surface density on efficiencies of hybridization-relevant electrocatalytic reactions, by using the previously described RuHex/$Fe(CN)_6^{3-}$ and MB//$Fe(CN)_6^{3-}$ systems. Based on this study, we also demonstrated a proof-of-concept electrocatalysis-based DNA sensor by elaborately adjusting DNA surface density (Scheme 1).

We employed thiolated capture probe DNA that could be self-assembled at the gold electrodes in a well-controlled manner. We first prepared a series of electrodes with different DNA surface densities (2.5 ± 0.2, 5.2 ± 0.3 and 20.0 ± 0.5 pmol cm^{-2}) in a similar way to the previously reported protocol, which was realized by varying probe concentration, timescale of self-assembly and/or ionic strength of the immobilization buffer.[13,20] The surface densities of thus prepared electrodes were quantitatively measured by using a RuHex probe and chronocoulometry.[20] We also evaluated the hybridization ability of these electrodes with chronocoulometry. Consistent with our previous report,[13,21] DNA hybridization was highly efficient (~80%) at low-density (LD) surfaces (2.5 pmol cm^{-2}), and much less efficient (~30%) at medium-density (MD) surfaces (5.2 pmol cm^{-2}), while only marginally possible (<5%) at high-density (HD) surfaces (20.0 pmol cm^{-2}).

In a solution containing 10 μM RuHex, we observed a pair of adsorption peaks at ≈ −0.21 V (E_{red-Ru} = −0.212 V; E_{ox-Ru} = −0.202 V) in cyclic voltammetry (CV), corresponding to the reduction and oxidation of RuHex electrostatically trapped within surface-confined DNA strands.[20,22,23] Interestingly, upon the addition of 2 mM $Fe(CN)_6^{3-}$ to the solution, we observed distinctly different phenomena for electrodes with HD and LD DNA monolayers (Fig. 1). For the HD surface (20 pmol cm^{-2}), a prominent electrocatalytic peak appeared in CV (electrocatalysis "ON"), which was characteristic of enhanced reduction peak and diminished oxidation peak. In contrast, while the whole CV shifted upward for the LD surface (2.5 pmol cm^{-2}), which was attributed to increased background arising due to the reduction current of $Fe(CN)_6^{3-}$, the peak shape changed little (electrocatalysis "OFF"). Of note, for convenience we arbitrarily set the "OFF" state as $I_{pa}/I_{pc} < 2$, where I_{pa} and I_{pc} stand for anodic and catholic peak currents, respectively.

In order to account for this marked difference, we started to investigate the effect of DNA surface density on the electrochemistry of $Fe(CN)_6^{3-}$ alone. We found that CVs of $Fe(CN)_6^{3-}$ were also highly dependent on DNA surface density. At the HD surface (20.0 pmol cm^{-2}), the CV of $Fe(CN)_6^{3-}$ were nearly irreversible, characteristic of large peak separation (ΔE = 0.751 V; E_{red-HD} = −0.254 V; E_{ox-HD} = 0.497 V; Fig. 1-S in the ESI†). In contrast, the redox behavior of $Fe(CN)_6^{3-}$ at the LD surfaces was much more reversible (ΔE = 0.171 V; E_{red-LD} = 0.152 V; E_{ox-LD} = 0.323 V). We attributed this difference to the coulombic repulsion between $Fe(CN)_6^{3-}$ and negatively charged DNA strands at the electrode surface. When the density of DNA was sufficiently high,

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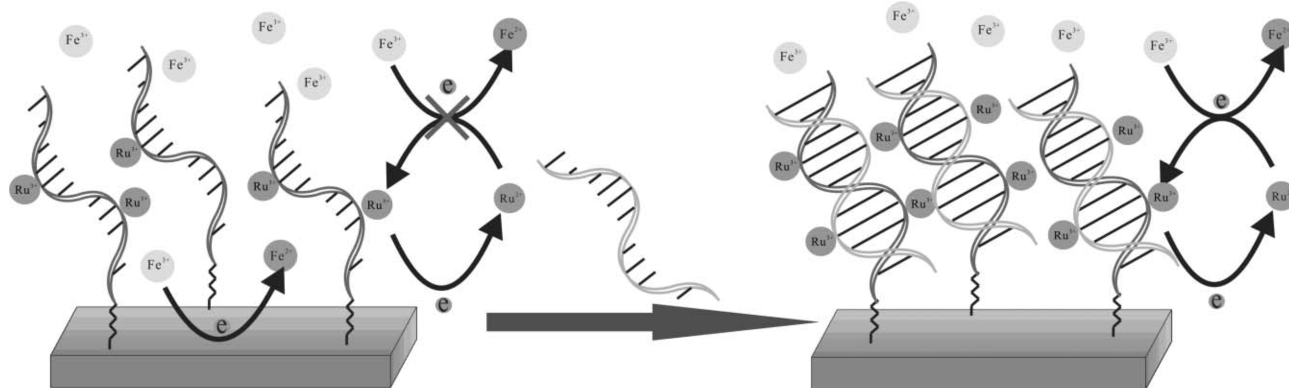
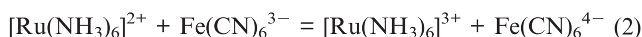


Scheme 1 Scheme for the "ON" and the "OFF" states of electrocatalysis associated with DNA hybridization.

it was difficult for $Fe(CN)_6^{3-}$ to approach the electrode, which significantly reduce the heterogeneous electron transfer rate. Of note, $E_{\text{red-Ru}}$ of $[Ru(NH_3)_6]^{3+}$ was at −0.212 V, which was higher than $E_{\text{red-HD}}$ while lower than $E_{\text{red-LD}}$.

Based on these observations, we propose a electron transfer kinetic-based mechanism. At the HD surface, since $Fe(CN)_6^{3-}$ is repelled and its reduction process of is very slow, $[Ru(NH_3)_6]^{3+}$ entrapped in the DNA film is reduced by the electrode prior to $Fe(CN)_6^{3-}$, and generated $[Ru(NH_3)_6]^{2+}$ ($E_{\text{red-Ru}} > E_{\text{red-HD}}$). Since $Fe(CN)_6^{3-}$ is a stronger oxidant than $[Ru(NH_3)_6]^{3+}$, $Fe(CN)_6^{3-}$ in the diffusion layer can oxidize $[Ru(NH_3)_6]^{2+}$ and regenerated $Ru(NH_3)_6^{3+}$ that can be reduced by the electrode again.[12] This electrocatalytic cycle is described as follows:

$$[Ru(NH_3)_6]^{3+} + e^- = [Ru(NH_3)_6]^{2+} \quad (1)$$



$$[Ru(NH_3)_6]^{2+} + Fe(CN)_6^{3-} = [Ru(NH_3)_6]^{3+} + Fe(CN)_6^{4-} \quad (2)$$

Note that there are a large amount of $Fe(CN)_6^{3-}$ in the diffusion layer, thus one RuHex molecule can eventually lead to the flow of many electrons through the electrode, as manifested by a significant increase of the reduction peak (termed the electrocatalytic peak). In contrast, at the LD surface, the reduction of $Fe(CN)_6^{3-}$ in the diffusion layer is kinetically faster than that of $Ru(NH_3)_6^{3+}$ ($E_{\text{red-Ru}} < E_{\text{red-LD}}$). Since $Fe(CN)_6^{3-}$ in the bulk solution cannot reach the electrode surface within the timescale of potential scan, $Fe(CN)_6^{3-}$ in the diffusion layer is transiently depleted, which makes it impossible to undergo electrocatalysis.

In order to confirm this mechanism, we further interrogated the MB/$Fe(CN)_6^{3-}$ system, which is analogous to the RuHex/$Fe(CN)_6^{3-}$ system except that MB binds to DNA mainly through intercalation in DNA double helixes.[11] In the absence of $Fe(CN)_6^{3-}$, there were a pair of CV peaks located around −0.2 V, corresponding to the reduction and oxidation of MB. Analogous to the RuHex/$Fe(CN)_6^{3-}$ system, in the presence of $Fe(CN)_6^{3-}$ there was prominent catalytic peak only at the HD surface (20.0 pmol cm^{-2}), while not at the LD surface (2.5 pmol cm^{-2}) (Fig. 2). This strongly suggested that electrocatalytic efficiencies were dependent on DNA surface density, while only minimally, if any, affected by the type of redox molecules (RuHex or MB) involved in the detection.

DNA hybridization is known to increase DNA surface density by bringing target DNA into the proximity of electrodes. Given that RuHex/$Fe(CN)_6^{3-}$ electrocatalysis occurring at DNA surfaces was highly dependent on the surface density of DNA, we proposed that sequence-specific DNA hybridization detection could be achieved by precise control of the DNA surface density. We

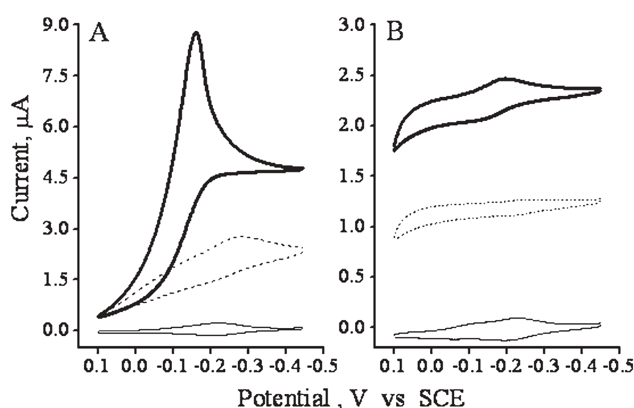


Fig. 1 CVs for the RuHex/$K_3Fe(CN)_6$ system. CVs of gold electrodes modified with (A) HD (20 pmol cm^{-2}) and (B) LD (2.5 pmol cm^{-2}) SH-DNA in 10 mM Tris buffer (pH 7.4) with 10 μM RuHex (solid line), 2 mM $K_3Fe(CN)_6$ (dashed line), and both 10 μM RuHex and 2 mM $K_3Fe(CN)_6$ (thick line). Scan rate: 50 mV s^{-1}.

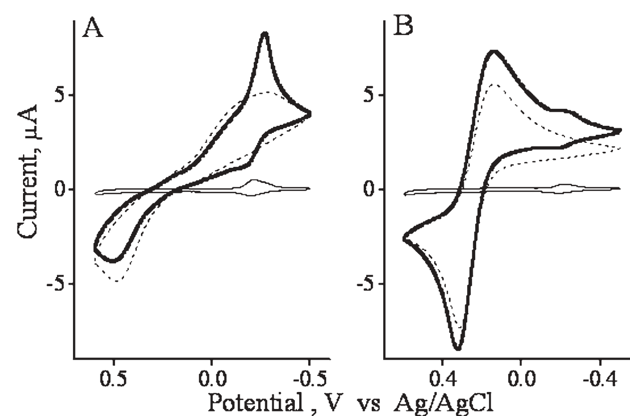


Fig. 2 CVs for the MB/$K_3Fe(CN)_6$ system. CVs of gold electrodes modified with (A) HD (20 pmol cm^{-2}) and (B) LD (2.5 pmol cm^{-2}) SH-DNA in 10 mM Tris buffer (pH 7.4) containing 6 μM MB (thin solid line), or 2 mM $K_3Fe(CN)_6$ (dashed line), or 6 μM MB + 2 mM $K_3Fe(CN)_6$ (thick solid line). Scan rate: 100 mV s^{-1}.

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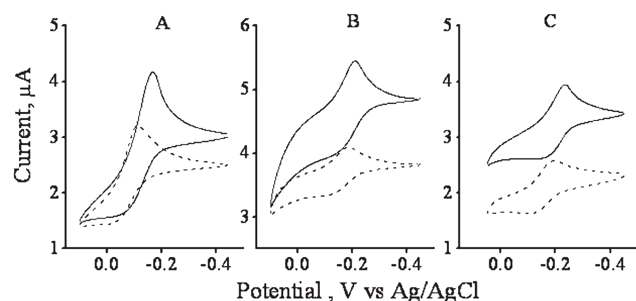

Fig. 3 DNA hybridization detection. CVs of DNA-modified electrodes in 10 mM Tris buffer (pH 7.4) with both 10 µM RuHex and 2 mM $K_3Fe(CN)_6$, before (dashed line) and after (solid line) hybridization with 100 nM target DNA. The surface densities were (A) 6.5 pmol cm^{-2}; (B) 5.2 pmol cm^{-2}, and (C) 2.5 pmol cm^{-2}, respectively. Scan rate: 50 mV s^{-1}.

Table 1 Comparison of hybridization efficiencies and electrochemical signal variation for electrodes with different DNA surface density

Surface density/ pmol cm^{-2}	Hybridization efficiency (%)	Signal variation (%)[a]
6.5 ± 0.4	29.1 ± 3.1	40.7 ± 3.9
5.2 ± 0.3	33.2 ± 2.3	100.4 ± 4.3
2.5 ± 0.2	75.5 ± 5.2	18.5 ± 3.0

[a] Signal variation is defined by $Q_{ds}/Q_{ss}-1$. Q_{ss} and Q_{ds} are integrated anodic charges before and after hybridization. The results were averaged from at least three independent experiments.

expected to find a critical surface density, below which electrocatalysis was "OFF" while above which electrocatalysis was "ON" (Scheme 1). After a few test and trial, we experimentally chose 5.2 pmol cm^{-2} as the optimal surface density. Initially, there was only minimal electrocatalysis at such a surface (electrocatalysis "OFF"); after hybridization with 100 nM complementary DNA, we observed significant enhancement of the reduction peak, indicating that the hybridization turned on electrocatalysis. We note that DNA hybridization led to a signal variation (reduction charge) larger than 100%, although hybridization efficiency was only ∼30% in this case (Fig. 3 and Table 1). In contrast, either higher or lower DNA surface density led to significantly smaller hybridization-induced signal variations. When the DNA surface density was only 2.5 pmol cm^{-2}, the increase of DNA surface density after hybridization was not sufficient to turn on electrocatalysis. Thus electrocatalysis was both "OFF" before and after hybridization, leading to a small signal variation (∼18%). When the DNA surface density reached 6.5 pmol cm^{-2}, electrocatalysis was already "ON" before detection, thus electrocatalysis was "ON" in both states (signal variation ∼40%).

We thus successfully demonstrated a proof-of-concept DNA hybridization sensor. This sensor has several interesting features. First, this is a truly label-free detection system. Since electrochemical signal arises due to the electrostatically bound RuHex and associated electrocatalysis, it is not necessary to conjugate either DNA targets or probes with electrochemical tags, which is expensive and not commercially available. Second, this sensor strategy exploits the inherent ON/OFF property of electrocatalysis, rather than simply relying on traditional affinity-based detection. Therefore it is potentially less susceptible to false-positive signals arising due to non-specific adsorption. Third, because the hybridization-induced ON/OFF change of electrocatalysis significantly improves the magnitude of signal variation, this sensor is potentially more sensitive than other electrocatalysis-based DNA sensors. Also of note, nanoparticle-based amplification has been well known to significantly improve the detection sensitivity.[13,24,25] We then reason that incorporation of nanoparticles coated with many signaling DNA strands in the present electrocatalysis system should further increase the detection sensitivity and probably set a new limit of detection. Such work is still under way in our laboratory.

In summary, we demonstrate that the RuHex/$Fe(CN)_6^{3-}$ electrocatalysis system is highly dependent on DNA surface density, and that DNA hybridization can "turn on" electrocatalysis at the appropriate DNA surface density. Based on these observations, we also provide a new way to detect DNA by using electrocatalysis. We expect that this convenient and sensitive sensing strategy can provide a highly promising approach for label-free detection of nucleic acids in various applications.

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