Electronic Supplementary Information (ESI) for the publication entitled:

Determination of the Binding Site Size of Hexaammineruthenium(III) inside Monolayers of DNA on Gold

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Cleaning of gold electrodes and determining their microscopic area

Gold electrodes (2 mm diameter) were electrochemically cleaned via reduction and oxidation scanning (10 cyclic scans at a scan rate of 0.1 V s^{-1}) in 0.5 M NaOH over the potential range of 0 and –1.6 V. They were subsequently polished on a pad coated with alumina suspension and washed with ethanol and ultrapure water. Electrochemical cleaning in acidic media was performed via potential cycling in 1 M H_2SO_4 (10 cyclic scans between –0.3 and 1.7 V at a scan rate of 0.3 V s^{-1}) and then in 1 M H_2SO_4 / 0.01 M KCl (10 cyclic scans between 0 and 1.7 V at a scan rate of 0.3 V s^{-1}).[1]

The microscopic area of the gold electrodes was determined based on the charge associated with the reduction of oxygen adsorbed on gold.[2-4] Oxygen was adsorbed onto the gold surface in a monoatomic layer through an anodic scan of the electrode potential from –0.35 to 1.5 V in 0.05 M H_2SO_4 (scan rate, 0.1 V s^{-1}). Upon scanning the potential back to –0.35 V, the oxygen monolayer was reduced and a sharp reduction peak appeared. The area under this peak (corrected for the capacitive current) represented the charge (in Coulombs) associated with the reduction of the surface oxide monolayer. The microscopic area of the gold electrodes was determined by dividing this charge by 482 μC cm^{-2}, the charge density corresponding to a complete monolayer of chemisorbed oxygen on gold.[5] The roughness factor for the prepared Au electrodes was typically 1.6.

Determination of the electron transfer rate constant

The electron transfer rate constant (k_{et}) of the MB redox process was calculated by the use of SWV according to the method described in reference 6. In this methodology, SWV curves are recorded over a range of frequencies and the "critical frequency", f_{max}, are obtained by plotting the ratio peak current/frequency (I_p/f) versus f, which typically shows a maximum at f_{max}. The value of f_{max} is used for the calculation of k_{et} according to the following relation:

$$k_{\mathrm{et}} = f_{\max} \times \omega_{max} \tag{1}$$

where $\omega_{\max}$ is the dimensionless electrode kinetic parameter at $f_{\max}$. The magnitude of $\omega_{\max}$, which depends on the electron-transfer coefficient and the normalized pulse amplitude (nE_{sw}), is determined by means of simulations.[6]

Binding kinetics of $RuHex^{3+}$ to surface-immobilized DNA

The adsorption of $RuHex^{3+}$ on surface-tethered DNA can be described by eq 2:

$$\frac{C^*_{\mathrm{Ru}}}{\Gamma_{\mathrm{Ru,e}}} = \frac{C^*_{\mathrm{Ru}}}{\Gamma_{\mathrm{Ru,s}}} + \frac{1}{K\Gamma_{\mathrm{Ru,s}}} \tag{2}$$

where $\Gamma_{\mathrm{Ru,e}}$ is the equilibrium quantity of $RuHex^{3+}$ adsorbed from a solution at concentration C^*_{Ru}, $\Gamma_{\mathrm{Ru,s}}$ is the saturation amount of $RuHex^{3+}$ adsorbed at higher concentrations, and K is the binding constant. The binding process may be written as[7]

$$[\mathrm{RuHex^{3+}}]_{\mathrm{soln}} + [(\mathrm{Tris^+})_n]_{\mathrm{surf}} \underset{k_{2\mathrm{b}}}{\overset{k_{2\mathrm{f}}}{\rightleftharpoons}} [\mathrm{RuHex^{3+}}]_{\mathrm{surf}} + [(\mathrm{Tris^+})_n]_{\mathrm{soln}} \qquad K_2 \tag{3}$$

in which $[\mathrm{RuHex^{3+}}]_{\mathrm{soln}} \equiv C^*_{\mathrm{Ru}}$, $[\mathrm{RuHex^{3+}}]_{\mathrm{surf}} \equiv \Gamma_{\mathrm{Ru,e}}$, $k_{2\mathrm{f}}$ and $k_{2\mathrm{b}}$ are second-order rate constants governing the cation-exchange reaction, and K_2 (= $k_{2\mathrm{f}}/k_{2\mathrm{b}}$) is the equilibrium constant of the reaction. In reaction 3, the cluster of n initially adsorbed $Tris^+$ molecules, $[(\mathrm{Tris^+})_n]_{\mathrm{surf}}$, that are substituted by each binding $RuHex^{3+}$ is treated as a single molecular unit. The equilibrium dynamics of the cation-exchange reaction in (3) are governed by the rate law given in eq 4

$$\frac{\mathrm{d}\Gamma_{\mathrm{Ru,t}}}{\mathrm{d}t} = k_{2\mathrm{f}} C^*_{\mathrm{Ru}} [\{(\mathrm{Tris^+})_n\}_{\mathrm{surf}}]_{\mathrm{t}} - k_{2\mathrm{b}} \Gamma_{\mathrm{Ru,t}} [(\mathrm{Tris^+})_n]_{\mathrm{soln}} \tag{4}$$

where the subscript t identifies time-dependent quantities. The amount of adsorbed $Tris^+$ clusters at any time, $[\{(\mathrm{Tris^+})_n\}_{\mathrm{surf}}]_{\mathrm{t}}$, can be written as

$$[\{(\mathrm{Tris}^+)_n\}_{\mathrm{surf}}]_\mathrm{t} = \Gamma_{\mathrm{Ru,s}} - \Gamma_{\mathrm{Ru,t}} \tag{5}$$

which can be combined with eq 2 to give

$$[\{(\mathrm{Tris}^+)_n\}_{\mathrm{surf}}]_\mathrm{t} = \left(\frac{KC^*_{\mathrm{Ru}} + 1}{KC^*_{\mathrm{Ru}}}\right)\Gamma_{\mathrm{Ru,e}} - \Gamma_{\mathrm{Ru,t}} \tag{6}$$

The rate law given in eq 4 thus becomes

$$\frac{\mathrm{d}\Gamma_{\mathrm{Ru,t}}}{\mathrm{d}t} = k_{2\mathrm{f}}C^*_{\mathrm{Ru}}\left[\left(\frac{KC^*_{\mathrm{Ru}} + 1}{KC^*_{\mathrm{Ru}}}\right)\Gamma_{\mathrm{Ru,e}} - \Gamma_{\mathrm{Ru,t}}\right] - k_{2\mathrm{b}}\Gamma_\mathrm{t}[(\mathrm{Tris}^+)_\mathrm{n}]_{\mathrm{soln}} \tag{7}$$

Integration of eq 7 for an initially prepared DNA-modified electrode placed in a $RuHex^{3+}$ solution yields

$$\ln\left(1 - \frac{\Gamma_{\mathrm{Ru,t}}}{\Gamma_{\mathrm{Ru,e}}}\right) = -k_{\mathrm{app}}t \tag{8}$$

where k_{app} is the apparent first-order rate constant given by

$$k_{\mathrm{app}} = k_{2\mathrm{f}}C^*_{\mathrm{Ru}} + k_{2\mathrm{b}}[(\mathrm{Tris}^+)_n]_{\mathrm{soln}} \tag{9}$$

By measuring the quantity of $RuHex^{3+}$ adsorbed from a $RuHex^{3+}$ solution onto a DNA monolayer at different times, one can calculate k_{app} using eq 8. For the desorption of $RuHex^{3+}$ into the pure supporting electrolyte from a DNA monolayer on which a quantity, $\Gamma_{\mathrm{Ru,0}}$, was adsorbed, and when the readsorption process can be neglected, we have[7]

$$\ln\left(\frac{\Gamma_{\mathrm{Ru,t}}}{\Gamma_{\mathrm{Ru,0}}}\right) = -k'_{\mathrm{app}}\,t \tag{10}$$

where k'_{app} is given by

$$k'_{\mathrm{app}} = k_{2\mathrm{b}}[(\mathrm{Tris}^+)_n]_{\mathrm{soln}} \tag{11}$$

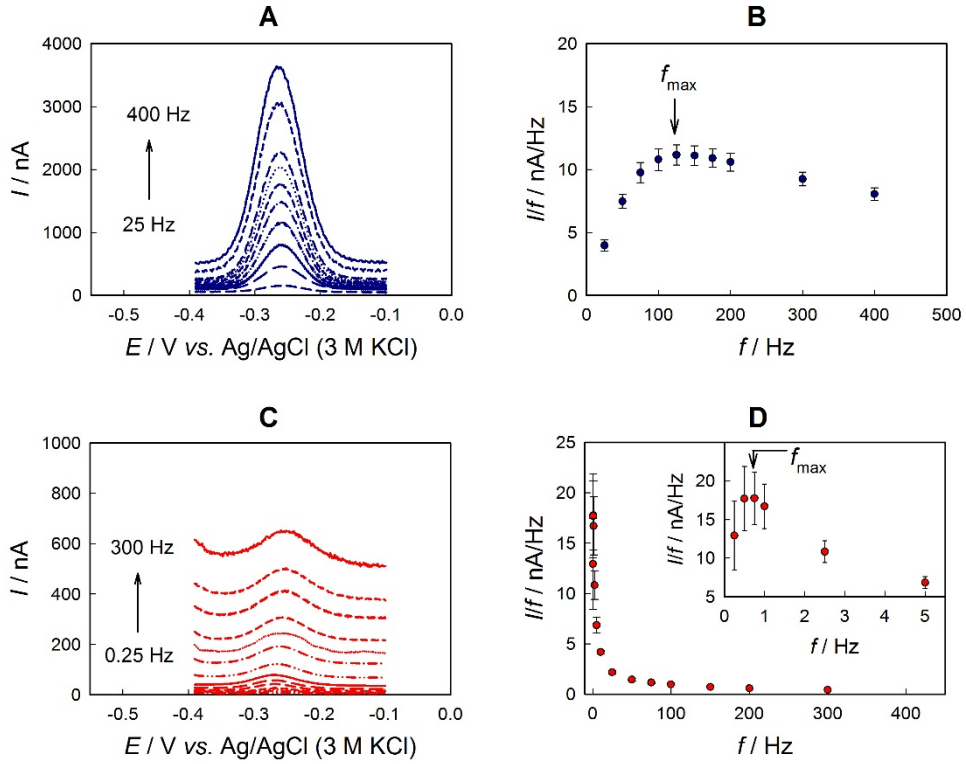

Figure S1. A and C are SWV curves at different *f* for gold-tethered DNA hybrids bearing MB at their proximal and distal ends, respectively. B and D show the corresponding plots of *I*/*f* versus *f*. Inset in D shows *I*/*f* for the frequency range below 5 Hz. k_{et} determined from f_{max} was 140 s^{-1} for the proximal MB and 0.7 s^{-1} for the distal MB. Both DNA monolayers were prepared under the same DNA immobilization conditions. Measurements were performed in 10 mM Tris buffer (pH 7.4) / 0.15 M NaCl.

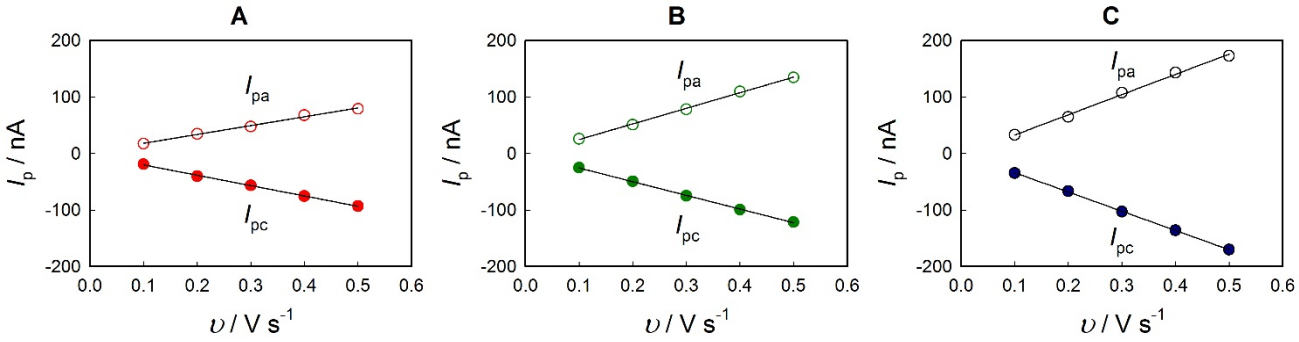

Figure S2. Plots of the CV peak currents versus υ for the electrodes modified with different amounts of the probe DNA and MB-labeled cDNA (with MB attached to the 5′-end). The DNA surface coverage was (A) 1.17, (B) 1.90, and (C) 3.24 pmol cm^{-2}. Voltammograms were recorded in 10 mM Tris buffer (pH 7.4) / 0.15 M NaCl.

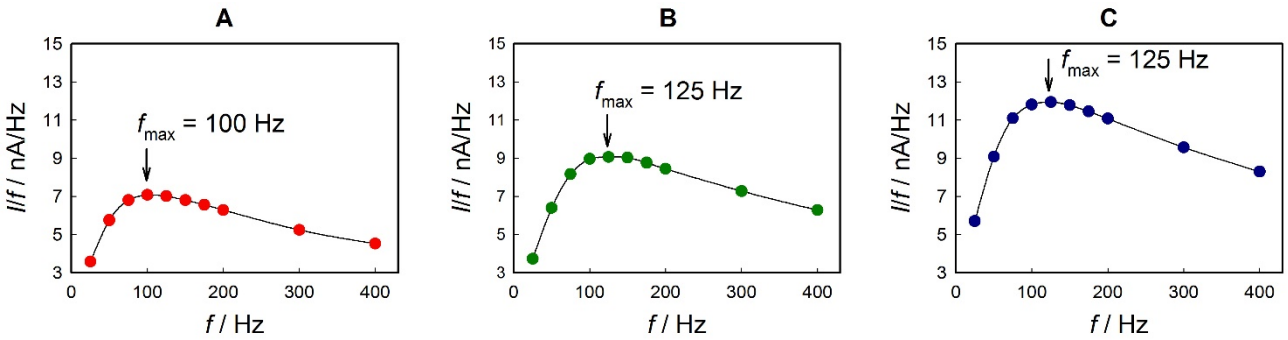

Figure S3. Plots of I/f versus f for SWV measurements recorded with electrodes that were modified with different amounts of the probe DNA and MB-labeled cDNA (with MB attached to the 5′-end). The DNA surface coverage was (A) 1.17, (B) 1.90, and (C) 3.24 pmol cm^{-2}. k_{et} determined based on the obtained f_{max} was (A) 112, (B) 140, and (C) 140 s^{-1}. Measurements were performed in 10 mM Tris buffer (pH 7.4) / 0.15 M NaCl. Solid lines are given as a guide to the eye only.

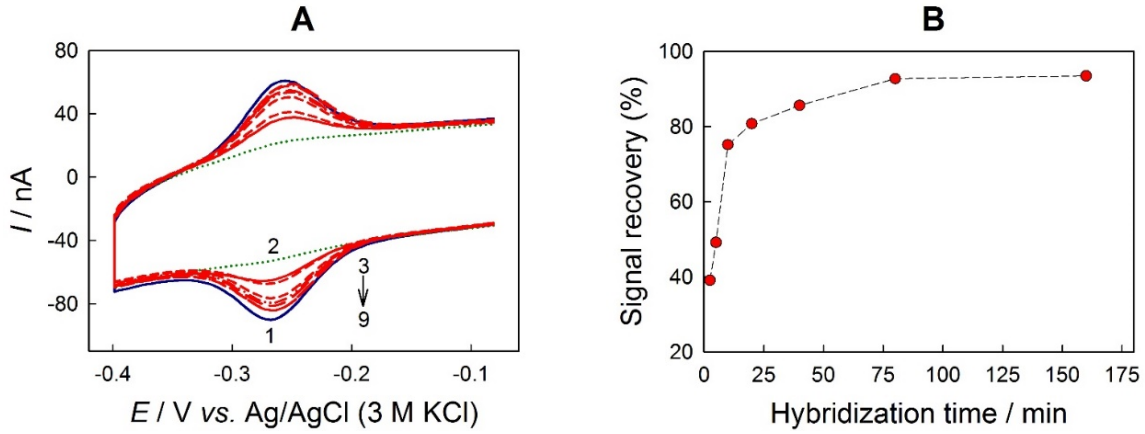

Figure S4. (A) CV curves of a monolayer of DNA duplexes (with MB attached to their proximal ends) before (1) and after (2) dehybridization. Curves 3-9 show cyclic voltammograms recorded after exposing the dehybridized monolayer to a solution containing 4 µM MB-labeled cDNA for 2.5−160 min, respectively. Voltammograms were recorded in 10 mM Tris buffer (pH 7.4) / 0.15 M NaCl at υ = 0.1 V s^{-1}. (B) The corresponding plot of signal recovery (calculated from the MB peak area) versus hybridization time. The dashed line is given as a guide to the eye only.

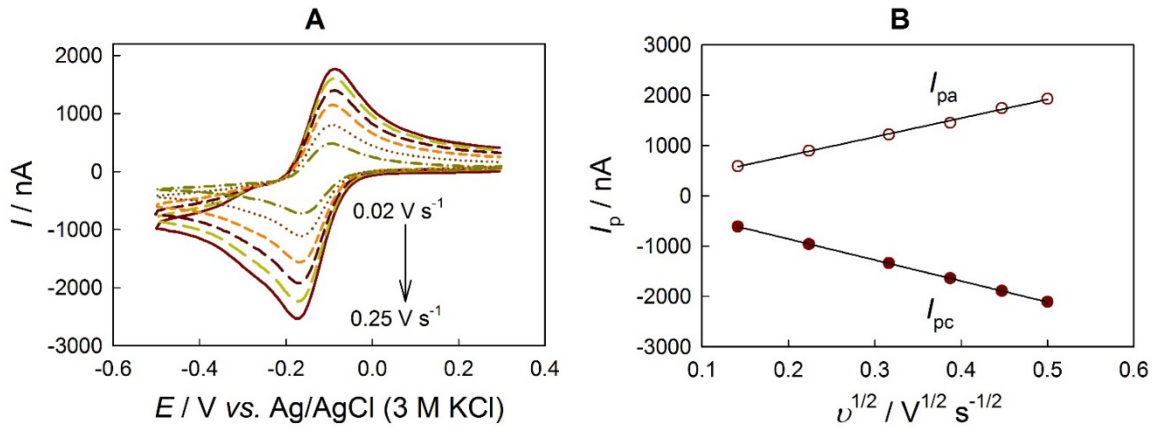

Figure S5. (A) CV curves recorded at different υ, and (B) the corresponding plot of the peak currents versus $\upsilon^{1/2}$ for a probe DNA-modified gold electrode. Measurements were performed in 10 mM Tris buffer (pH 7.4) containing 200 µM $RuHex^{3+}$.

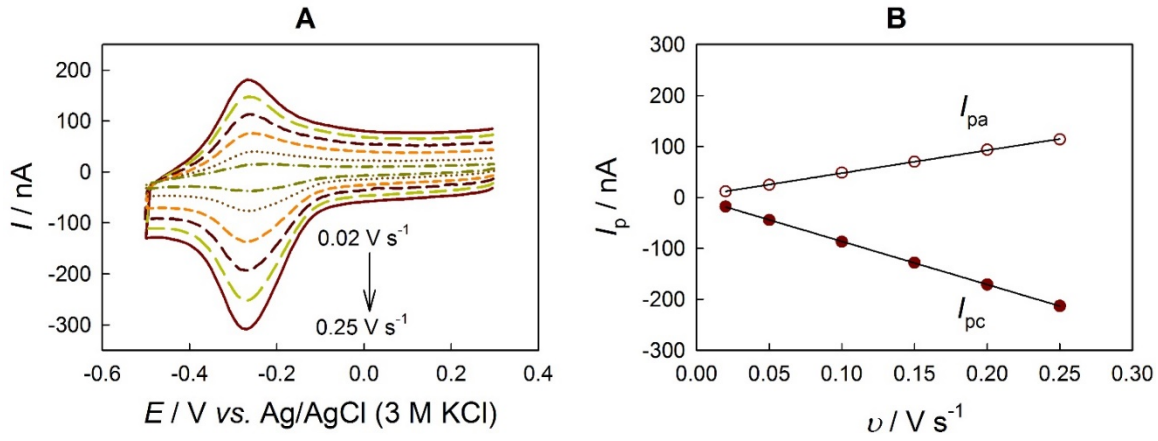

Figure S6. (A) CV curves at different υ, and (B) the corresponding plot of the peak currents versus υ for a probe DNA-modified gold electrode. Measurements were performed in 10 mM Tris buffer (pH 7.4) containing 2 µM $RuHex^{3+}$.

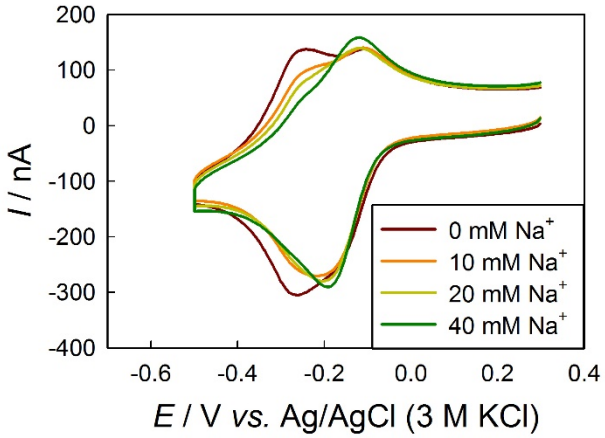

Figure S7. CV curves of a probe DNA-modified electrode recorded in 10 mM Tris buffer (pH 7.4) containing 20 µM $RuHex^{3+}$ and different concentrations of Na^+ cation (υ = 0.1 V s^{-1}).

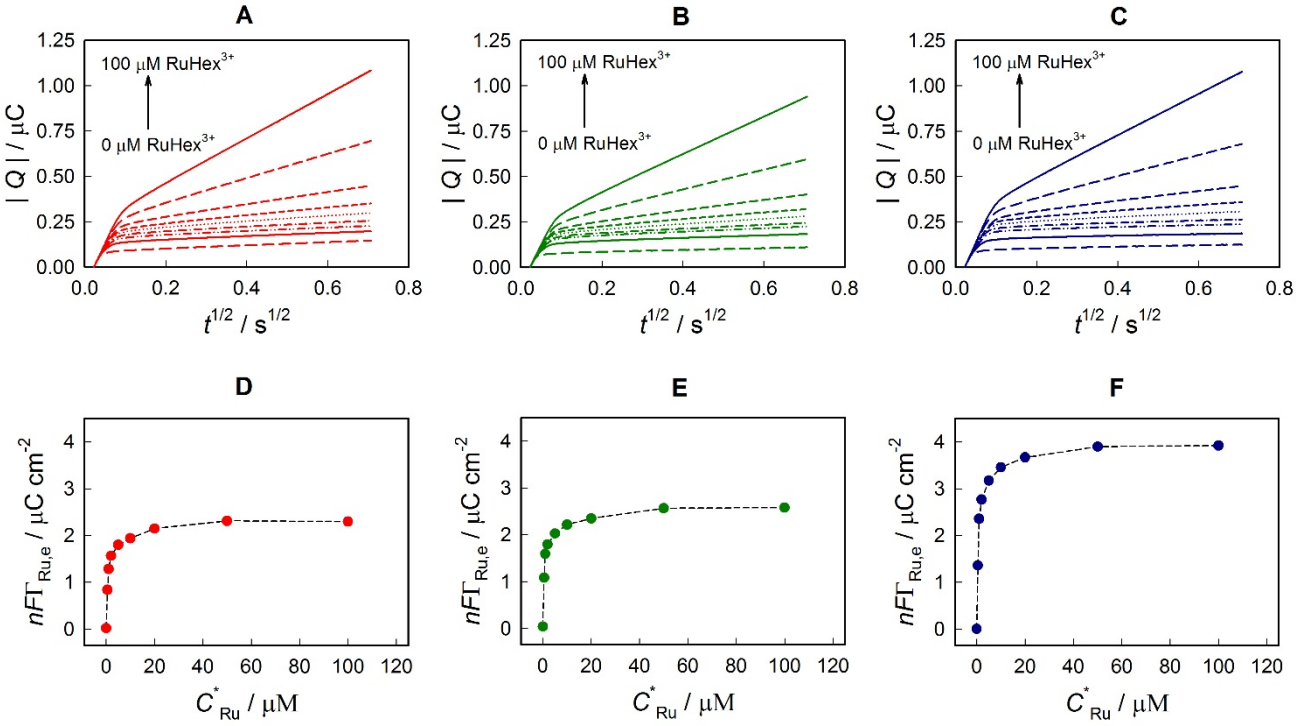

Figure S8. (A-C) Chronocoulometric curves, and (D-F) the corresponding adsorption isotherms obtained for the probe DNA-modified electrodes recorded in 10 mM Tris buffer (pH 7.4) containing different concentrations of $RuHex^{3+}$. Γ_{DNA} is (A and D) 1.17, (B and E) 1.90, and (C and F) 3.24 pmol cm^{-2}. Dashed lines in D-F are given as a guide to the eye only.

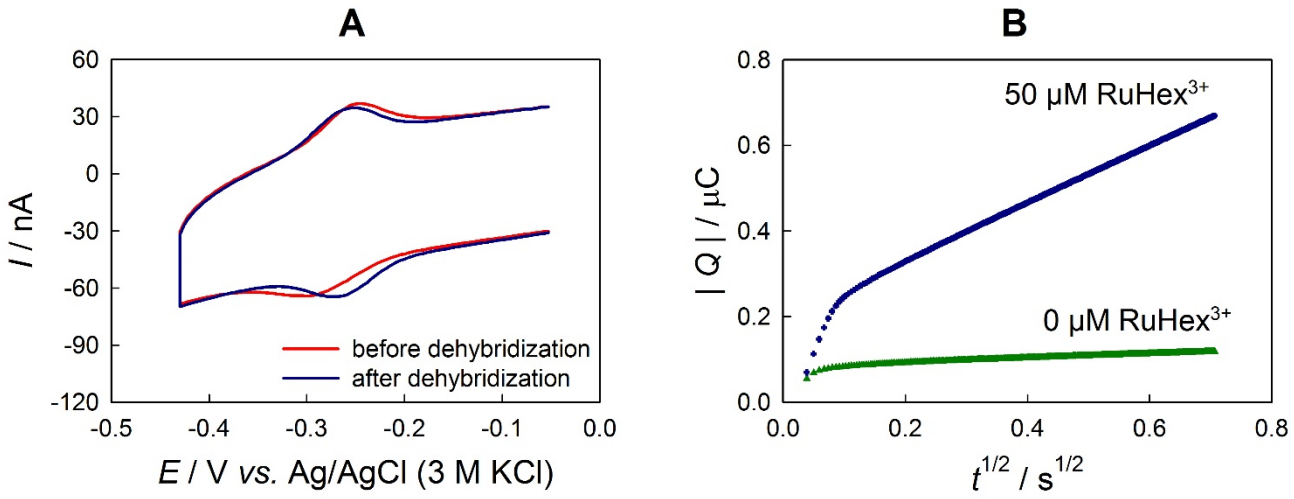

Figure S9. CV curves of an electrode modified with hybrids of the MB-labeled probe DNA and the unlabeled cDNA before and after dehybridization (υ = 0.1 V s^{-1}). The probe had a C_6-thiol modification at the 3′-end and an MB label at the 5′-end. The charges associated with the MB redox reaction were comparable in both hybridized and unhybridized states of the probe DNA and corresponded to a DNA surface coverage of 1.23 ± 0.05 pmol cm^{-2}. (B) Chronocoulometric curves of the dehybridized monolayer recorded in 10 mM Tris buffer (pH 7.4) in the absence and presence of 50 µM $RuHex^{3+}$, from the intercept of which $\Gamma_{Ru,s}$ was measured. The binding site size of $RuHex^{3+}$, *s*, inside this monolayer was calculated from the determined Γ_{DNA} and $\Gamma_{Ru,s,}$ and found to be 0.97 ± 0.05 nucleotide.

References

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