# Immobilization of DNA on an Aluminum(III) Alkanebisphosphonate Thin Film with Electrogenerated Chemiluminescent Detection

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# Immobilization of DNA on an Aluminum(III) Alkanebisphosphonate Thin Film with Electrogenerated Chemiluminescent Detection

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The immobilization of DNA on an electronically conductive surface is of interest both in studies of DNA itself and in various applications. For example, in scanning tunneling microscopy (STM) (or other scanning probe techniques), the DNA must be firmly affixed to a surface to minimize the effects of tip interactions during scanning.[1] Immobilization of DNA on an electrode is also used to produce biosensors, e.g., those that can recognize the base sequence on a complementary strand of DNA.[2] In this latter application, a single strand of DNA must be strongly attached to the electrode surface in a manner that still permits access to the bases and allows hybridization with a complementary DNA strand in solution.

The strategy employed in this study was to prepare, on a smooth gold substrate, a well-ordered film that contained metal centers, e.g., Al(III), that would interact strongly with the phosphate groups of DNA. Films of this type have been described previously.[3] Previous studies have also demonstrated binding of DNA to crystal (insulator) surfaces via the phosphate groups to $Ca^{2+}$ and $Mg^{2+}$ centers.[4] In the present study, the small amounts of DNA that were adsorbed on the surface were identified by intercalation into double-stranded (ds) DNA of a transition metal chelate, $Ru(phen)_3^{2+}$, which can be made to emit light when oxidized electrochemically in the presence of a suitable coreactant, in an approach previously used with solution phase DNA.[5] Film formation and DNA adsorption were also monitored with a quartz crystal microbalance (QCM).

The technique for the fabrication of the aluminum alkanebisphosphonate ($Al_2C_4BP$) film by sequential adsorption/reaction steps followed previous practice[3] and is illustrated in Figure 1a. 4-Mercaptobutylphosphonic acid (MBPA) was adsorbed on a gold film (2000 Å) sputtered on top of a 50 Å Cr layer on a silicon wafer.[6] The phosphonic acid-terminated surface was rinsed thoroughly with EtOH, dried in a stream of nitrogen, and then immersed in a 5 mM $Al(NO_3)_3$ solution. Following rinsing with water, it was immersed in 5 mM bisphosphonic acid, $H_2O_3P(CH_2)_4PO_3H_2$, rinsed with water, and then immersed in 5 mM $Al(NO_3)_3$ solution. As shown in previous studies of similar films, this produces a self-assembled film with a surface exposing many Al(III) binding sites.[7] Calf thymus ds-DNA was immobilized on the surface by immersing it in a solution of DNA (1.9 mM in nucleotide phosphate, NP) for 4 h (Figure 1b). The film was then rinsed three times with 4-mL portions of deionized water and then immersed for 4 h in either an aqueous 0.56 mM $Ru(phen)_3Cl_2$ solution or a 0.12 mM $Ru(phen)_3(ClO_4)_2$ solution in MeCN. As shown in previous studies, $Ru(phen)_3^{2+}$ associates with ds-DNA[8] and can be detected through its electrogenerated chemiluminescence (ECL).[5] Alternatively, the film could be soaked in a mixed ds-DNA (1.9 mM NP) and $Ru(phen)_3Cl_2$ (0.12 mM) solution for 4 h to produce the adsorbed layer.

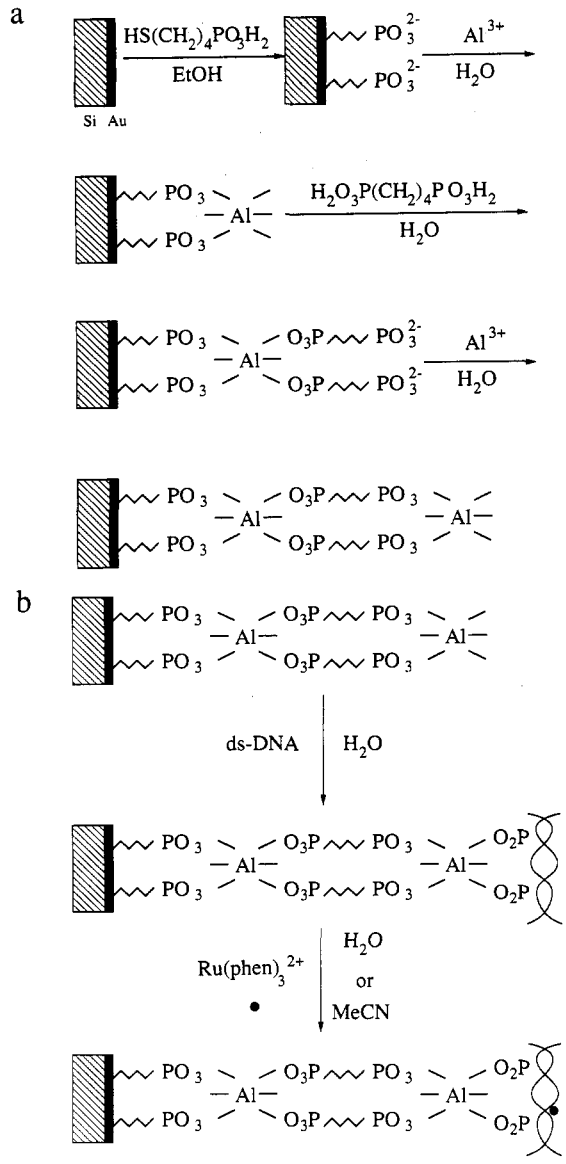


**Figure 1.** Schematic representation of (a) sequential steps in $Al_2(C_4BP)$ film formation on the gold surface supported on a silicon wafer and (b) immobilization of DNA on the film and interaction of $Ru(phen)_3^{2+}$ with the DNA.

ECL was produced by scanning the potential of the electrode following film formation, DNA adsorption, and $Ru(phen)_3^{2+}$ association, from 0 to 1.6 V vs a saturated calomel electrode (SCE) while it was immersed in a solution of 0.19 M phosphate buffer (pH 7) containing 0.13 M tri-*n*-propylamine (TPrA). Typical ECL transients, detected with a single-photon-counting apparatus, are shown in Figure 2. As proposed in previous studies,[5,9] emission arises from the energetic electron-transfer

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(6) Film preparation: Si wafers were soaked in trichloroethylene for 30 min, rinsed twice with 2-PrOH and then water, and dried in $N_2$ before sputtering of Cr and Au at $10^{-2}$ Torr. The Au surface was cleaned in hot chromic acid (saturated $K_2Cr_2O_7$ in 90% $H_2SO_4$) for 10 s, rinsed with copious amounts of water, and then immersed in 0.5 mM MBPA in EtOH for about 24 h. The surface was then thoroughly rinsed with EtOH, dried with $N_2$, and immersed alternately in 5 mM $Al(NO_3)_3$, 5 mM bisphosphonic acid, and 5 mM $Al(NO_3)_3$, taking 4 h for each immersion, with washing with water between each step.

(7) We have also prepared analogous films with Zr(IV) and La(III) centers. Preliminary experiments indicate a much smaller extent of immobilization of ds-DNA on these films.

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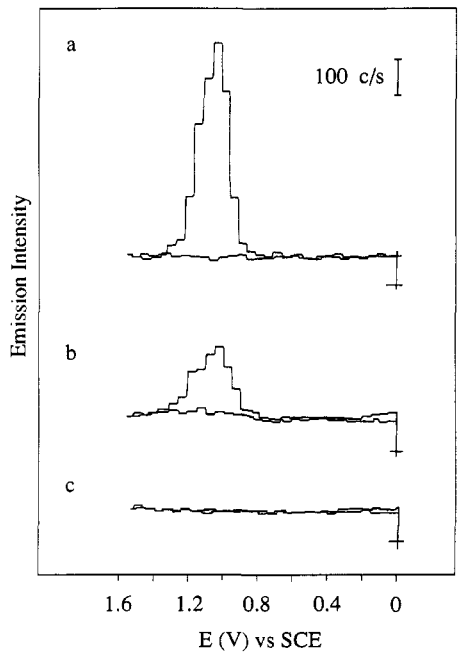


**Figure 2.** ECL emission–potential transients at the $Al_2(C_4BP)$/DNA-$Ru(phen)_3^{2+}$ electrode in 0.19 M phosphate buffer, pH 7, containing 0.13 M TPrA. (a) First, (b) second, and (c) third scans. (Scanning was halted and the solution stirred after each scan.) Scans were initiated at 0 V and were directed toward more positive potentials. Scan rate, 50 mV/s.

reaction between electrogenerated $Ru(phen)_3^{3+}$ and an intermediate in the oxidation of TPrA:

$$\text{DNA–Ru(phen)}_3^{2+}\text{ (adsorbed)} - e \rightarrow \text{DNA–Ru(phen)}_3^{3+} \quad (1)$$

$$CH_3CH_2CH_2\dot{N}Pr_2 - e \rightarrow CH_3CH_2CH_2\dot{N}Pr_2^{+} \quad (2)$$

$$CH_3CH_2CH_2\dot{N}Pr_2^{+} \rightarrow CH_3CH_2\dot{C}HNPr_2 + H^{+} \quad (3)$$

$$\text{DNA–Ru(phen)}_3^{3+} + CH_3CH_2\dot{C}HNPr_2 \rightarrow \text{DNA–Ru(phen)}_3^{2+*} + CH_3CH_2CHNPr_2^{+} \quad (4)$$

$$\text{DNA–Ru(phen)}_3^{2+*} \rightarrow \text{DNA–Ru(phen)}_3^{2+} + h\nu \quad (5)$$

The emission intensity decreased on a second scan, and none was seen on a third, suggesting loss of $Ru(phen)_3^{3+}$ from the film and diffusion into the bulk solution. In a control experiment, an electrode with a film of $Al_2C_4BP$ that had not been treated with DNA was soaked for 4 h in either an aqueous 0.56 mM $Ru(phen)_3^{2+}$ or a 0.12 mM $Ru(phen)_3^{2+}$ in MeCN solution and then rinsed with MeCN and water. This electrode showed no ECL emission upon potential sweep in the same TPrA solution described above, demonstrating that adsorption of $Ru(phen)_3^{2+}$ on the $Al_2C_4BP$ film does not occur.[10] The ability to generate $Ru(phen)_3^{3+}$ electrochemically in this ECL experiment demonstrates that the $Al_2C_4BP$ film and the DNA layer do not prevent heterogeneous electron-transfer reactions.

To obtain further evidence for $Al_2C_4BP$ film formation and DNA immobilization, experiments were undertaken with a gold-coated quartz crystal and a QCM.[3,11] The gold was repeatedly treated with hot chromic acid and then rinsed with water and EtOH until the surface was hydrophilic, as indicated by contact angle measurements. The crystal frequency was then measured in air during different stages of $Al_2C_4BP$ film formation,[6] after DNA adsorption, and after $Ru(phen)_3^{2+}$ association. The film was rinsed after each step with deionized water and dried in a stream of $N_2$ before measurement of the frequency. Results are shown in Table 1. Clearly, the crystal frequency decreases as the $Al_2C_4BP$ film forms and DNA and $Ru(phen)_3^{2+}$ are adsorbed on the surface, showing an increase of mass on the crystal during the different stages. The mass change, $\Delta m$, can be related to the frequency change, $\Delta f$, by the Sauerbrey equation:[11]

$$\Delta m = -[A(\rho_q\mu_q)^{1/2}/2F_0^2]\Delta f \quad (6)$$

where $F_0$ is the fundamental frequency of the unloaded crystal (6 MHz), $A$ is the electrode area (0.159 $cm^2$), $\rho_q$ is the density of quartz (2.65 $g/cm^3$), and $\mu_q$ is the shear modulus of quartz ($2.95 \times 10^{11}$ $dyn/cm^2$). With these constants, eq 6 becomes

$$\Delta m\ (ng) = -1.95\Delta f\ (Hz) \quad (7)$$

The mass changes calculated in this way are also given in Table 1. These can be converted to approximate surface concentrations, $\Gamma$, assuming a roughness factor of 2 (i.e., total surface area of both sides of the quartz crystal of 0.6 $cm^2$).[3] ECL could also be detected from both gold surfaces of the quartz crystal when used as a substrate for $Al_2C_4BP$ film formation, DNA adsorption, and $Ru(phen)_3^{2+}$ association and then scanned in the TPrA solution.

We have demonstrated that an electrode surface can be designed which immobilizes DNA while not adsorbing a detector molecule, $Ru(phen)_3^{2+}$. Moreover, ds-DNA on the surface can be detected by electrogenerated chemiluminescence of associated $Ru(phen)_3^{2+}$. Characterization of the adsorbed DNA by transmission electron microscopy and atomic force microscopy is currently being investigated. We have also found that single-stranded DNA can be immobilized on the $Al_2C_4BP$ film surface and then hybridized with complementary DNA in solution with detection of the ds-DNA produced by ECL. Details of these experiments will be reported elsewhere.

**Table 1.** Frequency and Mass Changes on a QCM Plate for Film Growth, DNA Immobilization, and $Ru(phen)_3^{2+}$ Binding

| | $\Delta f^g$ (Hz) | $\Delta m$ (ng) | $10^9\Gamma^h$ (mol/cm²) |
|---|---|---|---|
| after immersion in[a] | | | |
| MBPA[b] | −70 | 137 | 1 |
| $Al(NO_3)_3$[c] | −27 | 53 | 1[i] |
| $C_4BPA$[d] | −145 | 283 | 2 |
| $Al(NO_3)_3$[c] | −25 | 49 | 1[i] |
| ds-DNA[e] | −24 | 47 | 0.3[j] |
| $Ru(phen)_3^{2+}$[f] | −18 | 35 | 0.1 |

[a] Immersions were sequential in order given from top to bottom; bare surface was gold; bare crystal frequency, 6.011 329 MHz. [b] 0.5 mM MBPA in EtOH for ~24 h. [c] 5 mM aqueous $Al(NO_3)_3$ for ~4 h. [d] 5 mM phosphonic acid for 4 h. [e] Calf thymus DNA (3.8 mM NP) for 4 h. [f] 0.24 mM $Ru(phen)_3^{2+}$ in MeCN for 4 h. [g] The variation and drift of the signal over the series of measurements was about ±1 Hz. [h] Assuming roughness factor for gold of 2 (total surface area, 0.6 $cm^2$). For comparison, for a close-packed monolayer of MBPA, $\Gamma \approx 0.6 \times 10^{-9}$ mol/$cm^2$. [i] Assuming an $Al(H_2O)_3^{3+}$ unit is adsorbed. [j] Corresponding to number of moles of nucleotide phosphate per $cm^2$.

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