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

## Xiao-Hong Xu and Allen J. Bard*

*Contribution from the Department of Chemistry and Biochemistry, The University of Texas at Austin, Austin, Texas 78712*

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**Abstract:** Immobilization of single-stranded (ss) DNA on an electrode covered with an aluminum alkanebisphosphonate film by immersion in an ss-DNA solution was demonstrated with ss-DNA labeled with $Ru(bpy)_3^{2+}$. This was detected by observing the electrogenerated chemiluminescence (ECL) produced upon oxidation in a solution containing tri-*n*-propylamine. Surface hybridization was shown by immobilization of unlabeled ss-DNA, followed by exposure to a complementary labeled strand of DNA to produce $Ru(bpy)_3^{2+}$-labeled double-stranded (ds) DNA on the surface. The extent of DNA hybridization was determined by ECL. Hybridization of unlabeled DNA, e.g., poly(dA) and poly(dT), could be detected by treatment with $Ru(phen)_3^{2+}$ and ECL of this species associated with the ds-DNA. In a similar way, an eight-base single strand of DNA was determined by immobilizing it on the film followed by hybridization of the eight-base complementary strand. The surface-immobilized eight-base pair ds-DNA was also detected by observing the ECL of intercalated $Ru(phen)_3^{2+}$.

The immobilization and hybridization of DNA on a self-assembled thin film is of interest both in studies of molecular recognition of DNA and in various applications.[1-3] Apart from providing a predictable surface that binds a complementary strand of DNA on substrates for studying DNA double-helix formation and its interactions with proteins and other molecules,[4] a film with single-stranded (ss) DNA can form the basis of a DNA biosensor, e.g., by serving as a designed surface modified electrode. DNA diagnostics has become an important area in molecular biology and biotechnology studies, with applications to the determination of disease-causing and food-contaminating organisms and in forensic and environmental investigations. However, traditional methods for DNA sequencing have several disadvantages, e.g., they are labor intensive, expensive, time consuming, and difficult to automate.[5,6] The development of new DNA biosensors has involved the application of optical methods (e.g., luminescence, ellipsometry, and pseudo-Brewster angle reflectometry), piezoelectric devices (e.g., surface acoustic wave, quartz crystal microbalance), and electrochemical techniques (e.g., cyclic voltammetry and square wave voltammetry).[5,7] Electrogenerated chemiluminescence (ECL) has also been applied to DNA analysis, e.g., of DNA produced by the polymerase chain reaction by capturing $Ru(bpy)_3^{2+}$-labeled DNA on magnetic beads, transporting the beads to an electrochemical cell, and detecting emission under electrochemical excitation.[8,9]

In a previous study, we demonstrated that an aluminum(III) alkanebisphosphonate film can be used to immobilize calf thymus double-stranded (ds) DNA based on the interaction of the film metal center ($Al^{3+}$) with the phosphate group of DNA and that the immobilized DNA could be determined by ECL detection upon oxidation of a solution of $Ru(phen)_3^{2+}$ intercalated with DNA and tri-*n*-propylamine.[10] The technique for the fabrication of the aluminum alkanebisphosphonate, $Al_2(C_4BP)$, film by sequential adsorption/reaction steps has been described previously.[10,11]

We report here that the film can also be used to immobilize ss-DNA (e.g., poly(dA), poly(dT), poly(dC), and eight-base and 30-base synthesized DNA chains). After the immobilization, the film can be employed to recognize a complementary strand of DNA in a solution followed by ECL detection. The strategy employed is shown in Figure 1.

## Experimental Section

**Apparatus.** The Plexiglas cell designed for ECL and electrochemical studies of the film immobilized on the gold surface supported on the silicon wafer was described previously.[10,12] A saturated calomel reference electrode (SCE) and a platinum wire counterelectrode were used for all measurements. The electrochemical measurements coupled with ECL experiments were carried out with a Model 175 universal programmer, a Model 173 potentiostat (Princeton Applied Research, PAR, Princeton, NJ), and an Omnigraphic 2000 x-y recorder (Houston Instruments, Houston, TX). The ECL emission was detected with a Model C1230 single-photon-counting system (Hamamatsu Corp., Bridgewater, NJ) utilizing a Hamamatsu R928P PMT, cooled to −20

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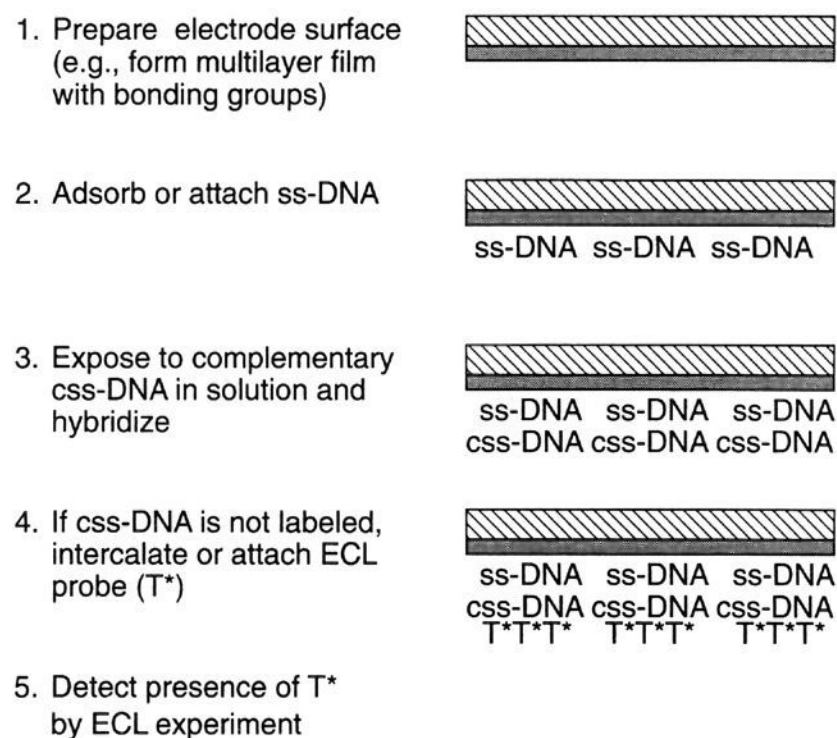(12) Xu, X.-H.; Bard, A. J. *Langmuir* **1994,** *10,* 2409.

1. Prepare electrode surface (e.g., form multilayer film with bonding groups)

2. Adsorb or attach ss-DNA

ss-DNA  ss-DNA  ss-DNA

3. Expose to complementary css-DNA in solution and hybridize

ss-DNA  ss-DNA  ss-DNA
css-DNA css-DNA css-DNA

4. If css-DNA is not labeled, intercalate or attach ECL probe (T*)

ss-DNA  ss-DNA  ss-DNA
css-DNA css-DNA css-DNA
T*T*T*  T*T*T*  T*T*T*

5. Detect presence of T* by ECL experiment

**Figure 1.** Strategy for making a DNA probe by surface attachment of DNA for detection by ECL.

°C in a Model TE 308 TSRF cooler controller (Products for Research Inc., Danvers, MA). The meter output was fed into the y-axis of the x-y recorder, and the signal from the potentiostat was fed into the x-axis to afford ECL intensity versus potential displays. Solution analysis by ECL was carried out with a QPCR analyzer (Perkin-Elmer, Norwalk, CT). An MRC (Materials Research Corporation, Orangeburg, NY) Model 8620 sputtering system at $10^{-2}$ Torr, with an RF power of 150 W and RF peak to peak voltage of 1.8 KV, was used to sputter gold (99.999%) on silicon wafers.

**Chemicals and Materials.** Polydeoxyadenylic acid (poly(dA)), polythymidylic acid (poly(dT)), polydeoxycytidylic acid (poly(dC)), and calf thymus (CT) ss-DNA and ds-DNA were purchased from Sigma Chemical Co. (St. Louis, MO) and used without further purification. The eight-base DNA samples, 5'GCCGCCAT3' and 5'ATGGCGGC3', were synthesized by an Applied Biosystems 381A DNA synthesizer. The DNA solutions were prepared by dissolving the resuspended ss-DNA in a 5 mM tris buffer containing 50 mM NaCl (pH 7). The 30-

base ss-DNA samples, λ-1 DNA (5'GAAAATGTGCTGACCGGA-CATGAAAATGAG3'), λ-1 DNA tagged with $Ru(bpy)_3^{2+}$ (5'Ru(bpy)$_3^{2+}$-GAAAATGTGCTGACCGGACATGAAAATGAG3'), and a complementary strand λ-1c DNA (5'CTCATTTTCATGTCCGGTCAGCA-CATTTTC3'), were obtained from Perkin-Elmer and diluted with 5 mM tris buffer containing 50 mM NaCl (pH 7).

Trichloroethylene (99.6%, Baker), 2-propanol (99.9%, Baker), tripropylamine (TPrA) (98%, Aldrich), $Ru(bpy)_3Cl_2\cdot6H_2O$ (Aldrich), $Ru(phen)_3Cl_2\cdot6H_2O$ (Aldrich), ethyl alcohol (200 proof, AAPER Alcohol), $Al(NO_3)_3\cdot9H_2O$ (Spectrum Chemical MFG), $K_2Cr_2O_7$ (Fisher), $NaH_2PO_4$ (Baker), and tris(hydroxymethyl)aminomethane (Sigma) were used as received without purification. Bisphosphonic acid, $H_2O_3P(CH_2)_4$-$PO_3H_2$ ($C_4BPA$), and 4-mercaptobutylphosphonic acid (MBPA) were obtained from the Mallouk group.[11] The assay buffer for ECL experiments contained 0.13 M TPrA and 0.19 M phosphate buffer, prepared by dissolving TPrA into a $NaH_2PO_4$ solution and adjusting the pH to 7 with 1 M NaOH. Deionized water from a Millipore Milli-Q (18 MΩ-cm) system was used to prepare all aqueous solutions and to rinse the electrode surface.

**Film Preparation.** The anchoring films were produced as shown in Figure 2a. The silicon wafers were soaked in trichloroethylene for 30 min, rinsed twice with 2-propanol, rinsed with excess amounts of deionized water, and then dried with a stream of dry nitrogen. The clean silicon wafers were primed with a 50-Å chromium layer followed by deposition of a 2000-Å gold layer. Chromium and gold targets (99.999%) were used to sputter the films onto the wafers in a MRC Model 8620 system at $10^{-2}$ Torr.

The gold surface supported on the silicon wafers was cleaned with hot chromic acid (saturated $K_2Cr_2O_7$ in 90% $H_2SO_4$) for ~10 s and then rinsed with copious amounts of water. This process was repeated until the surface contact angle with water was less than 15°. The clean Au was then immediately soaked in the anchoring agent, 0.5 mM 4-mercaptobutylphosphonic acid (MBPA) solution in absolute ethanol for ~24 h. The phosphonic acid terminated surface was thoroughly rinsed with ethanol, dried with a stream of $N_2$, and then immersed alternately in 5 mM $Al(NO_3)_3$, 5 mM bisphosphonic ($H_2O_3P(CH_2)_4$-$PO_3H_2$), and 5 mM $Al(NO_3)_3$ aqueous solution, taking 4 h for each immersion, with washing with water between each step to produce the film, designated $Al_2(C_4BP)$.

**Single-Stranded DNA Immobilization and Hybridization.** The λ-1 tagged ss-DNA (i.e., labeled with $Ru(bpy)_3^{2+}$) was immobilized

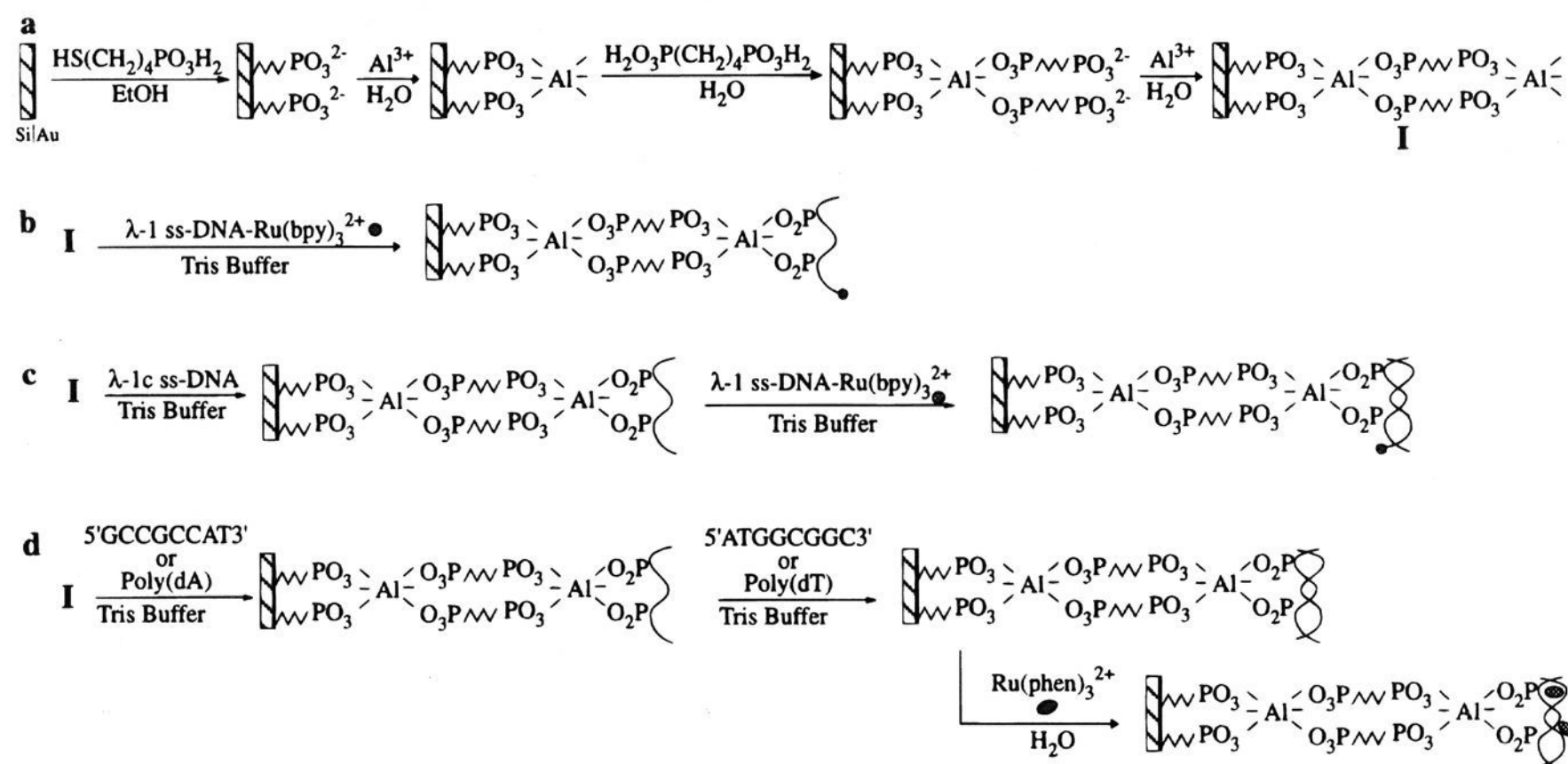**Figure 2.** Schematic representation of (a) sequential steps in $Al_2(C_4BP)$ film formation on a gold surface supported on a silicon wafer;[10] (b) immobilization of ss-DNA tagged with $Ru(bpy)_3^{2+}$ on the film; (c) immobilization of ss-DNA on the film followed by hybridization of a complementary strand of DNA tagged with $Ru(bpy)_3^{2+}$; (d) immobilization of poly(dA) on the film, hybridization with poly(dT), and then interaction of Ru-(phen)$_3^{2+}$ with the ds-DNA, poly(dA)·poly(dT), or immobilization of eight-base ss-DNA on the film, hybridization of the eight-base complementary strand of DNA, and then interaction of Ru(phen)$_3^{2+}$ with the ds-DNA.
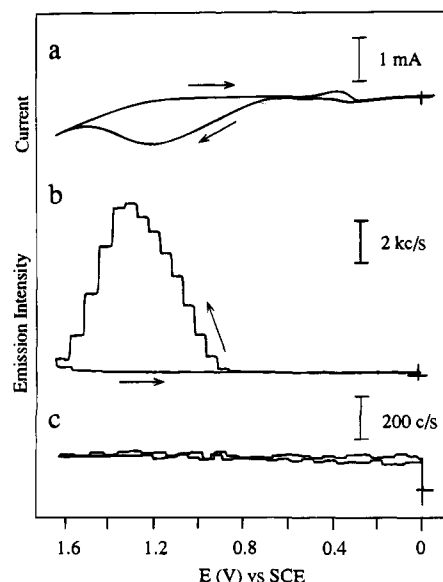
**Figure 3.** (a) Cyclic voltammogram and (b) emission-potential transient of the $Al_2(C_4BP)/\lambda$-1 ss-DNA-Ru(bpy)$_3^{2+}$ electrode in 0.19 M phosphate buffer/0.13 M TPrA, pH 7 and (c) emission-potential transient of the $Al_2(C_4BP)/\lambda$-1 ss-DNA electrode in the same solution. The electrodes were prepared as described in the text with immersion in a 1.38 $\mu$M $\lambda$-1 ss-DNA-Ru(bpy)$_3^{2+}$ or $\lambda$-1 ss-DNA solution for ~4 h. In each case, the potential was scanned from 0 to 1.60 V at scan rate, 50 mV/s. The staircase shape of the emission is the result of integration of the photo flux in the single-photon-counting system. (c/s = photomultiplier counts per second.)

on the aluminum phosphate film by immersing the film in the DNA solution (Figure 2b). The amount of immobilized DNA-Ru(bpy)$_3^{2+}$ on the surface was determined by ECL resulting from the oxidation of Ru(bpy)$_3^{2+}$ and TPrA in a solution.

After the unlabeled $\lambda$-1c ss-DNA was immobilized on the film, the film was incubated in a complementary strand $\lambda$-1 labeled ss-DNA solution at 60 °C for 5 min and then cooled to the room temperature gradually; during this cooling period, the ss-DNA hybridized with the complementary strand DNA (Figure 2c). The hybridized DNA-Ru-(bpy)$_3^{2+}$ on the film was detected by ECL as described above.

Poly(dA) was immobilized on the film by soaking the film in a poly-(dA) solution. After the immobilization, poly(dT) was hybridized with poly(dA) to produce poly(dA)·poly(dT) ds-DNA on the surface by incubating the film in a poly(dT) solution at 70 °C for 5 min and then cooling to room temperature gradually (Figure 2d). To intercalate Ru-(phen)$_3^{2+}$ into the ds-DNA (poly(dA)·poly(dT)), the $Al_2(C_4BP)$/poly-(dA)·poly(dT) film was treated with a Ru(phen)$_3^{2+}$ solution.[10] The hybridized poly(dA)·poly(dT)-Ru(phen)$_3^{2+}$ on the surface was determined by ECL based on the oxidation of Ru(phen)$_3^{2+}$ and TPrA in the solution. A similar procedure was employed to immobilize the eight-base ss-DNA, 5'GCCGCCAT3', on the film, followed by hybridization with the complementary eight-base ss-DNA, 5'ATGGCGGC3', to produce ds-DNA, which was then intercalated with Ru(phen)$_3^{2+}$.

## Results and Discussion

**Immobilization and Detection of Ru(bpy)$_3^{2+}$-Labeled 30-Base ss-DNA.** The aluminum phosphonate film, $Al_2(C_4BP)$, prepared as described above, was immersed in a 1.38 $\mu$M solution of $\lambda$-1 30-base ss-DNA (tagged with Ru(bpy)$_3^{2+}$) for ~2 h. This was thoroughly washed with water and transferred to the ECL cell where it was employed as a working electrode for an ECL experiment in a 0.19 M phosphate buffer (pH 7) containing 0.13 M TPrA. Cyclic voltammograms and emission transients were obtained by scanning the potential of the electrode from 0 to 1.6 V vs SCE. A representative voltammogram and the emission detected with a single-photon-counting system are shown in Figure 3. The broad oxidation
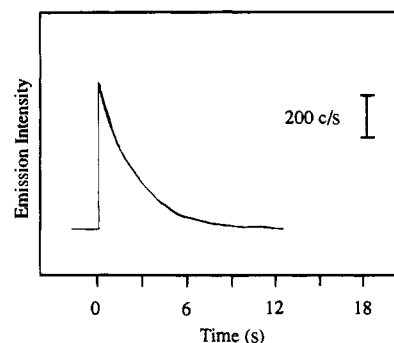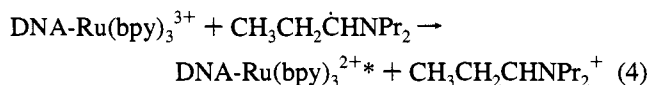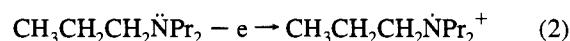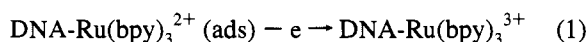


**Figure 4.** Emission-time transient for the $Al_2(C_4BP)/\lambda$-1 ss-DNA-Ru-(bpy)$_3^{2+}$ electrode in 0.19 M phosphate buffer/0.13 M TPrA, pH 7, when the potential was stepped from 0 to 1.5 V.

wave at ~1.2 V observed in the voltammogram (Figure 3a) arises from the oxidation of TPrA, Ru(bpy)$_3^{2+}$, and the Au substrate; the small reduction wave at ~0.35 V is from the reduction of gold oxide. The ECL emission from the $Al_2(C_4$-BP)$/\lambda$-1 ss-DNA-Ru(bpy)$_3^{2+}$ electrode (Figure 3b) demonstrates that the $\lambda$-1 30-base ss-DNA-Ru(bpy)$_3^{2+}$ was immobilized on the film. The decay of the light intensity ($I$) as a function of time ($t$) was also investigated with the single-photon-counting system. The light intensity detected by the photomultiplier tube, when the potential was stepped from 0 to 1.50 V, decreased with time (Figure 4). The decay of intensity with time suggests desorption of ss-DNA-Ru(bpy)$_3^{2+}$ from the electrode surface upon oxidation or irreversible decomposition of the emitter or, more probably, the destruction of the film during the potential step. Thiolate films on Au are known to be unstable at positive potentials. As proposed in previous studies,[13,14] emission results from the energetic electron-transfer reaction between electrogenerated Ru(bpy)$_3^{3+}$ and an intermediate in the oxidation of TPrA:

$$DNA\text{-}Ru(bpy)_3^{2+} (ads) - e \rightarrow DNA\text{-}Ru(bpy)_3^{3+} \quad (1)$$

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

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

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

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

No ECL emission was observed from a film prepared by immersing $Al_2(C_4BP)$ in solutions of unlabeled ss-DNA (either 1.38 $\mu$M $\lambda$-1 30-base ss-DNA or 0.37 $\mu$M $\lambda$-1c 30-base ss-DNA solutions, Figure 3c). A blank control experiment with the aluminum phosphonate film immersed in a 304 nM Ru(bpy)$_3^{2+}$ solution for ~4 h shows a negligible ECL signal, indicating that Ru(bpy)$_3^{2+}$ does not adsorb on the film and oxidize. This confirms that only a negligible amount of the underlying Au remains uncovered, since adsorption of Ru(bpy)$_3^{2+}$ and ECL on Au electrodes have previously been observed.[12]

**Hybridization of 30-Base ss-DNA.** After the immobilization of unlabeled $\lambda$-1c 30-base ss-DNA on the $Al_2(C_4BP)$ film described above, the film was immersed in a solution of 1.38 $\mu$M complementary ss-DNA tagged with Ru(bpy)$_3^{2+}$ ($\lambda$-1 30-base ss-DNA-Ru(bpy)$_3^{2+}$). The film in the solution was gradually heated to 60 °C in a water bath, incubated at 60 °C

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**Table 1.** Summary of ECL Data from Detection of ss-DNA Immobilization and Hybridization

| electrode | ECL peak intensity[a] | (c/s) |
|---|---|---|
| $Al_2(C_4BP)/\lambda$-1c ss-DNA/$\lambda$-1 ss-DNA-Ru(bpy)$_3^{2+}$ | very strong | ~42600 |
| $Al_2(C_4BP)/\lambda$-1 ss-DNA/$\lambda$-1 ss-DNA-Ru(bpy)$_3^{2+}$ | none | 0 |
| $Al_2(C_4BP)/poly(dA)/poly(dT)/Ru(phen)_3^{2+}$ | strong | ~781 |
| $Al_2(C_4BP)/poly(dA)/poly(dA)/Ru(phen)_3^{2+}$ | none | 0 |
| $Al_2(C_4BP)/poly(dA)/poly(dC)/Ru(phen)_3^{2+}$ | none | 0 |
| $Al_2(C_4BP)/5'GCCGCCAT3'/5'ATGGCGGC3'/Ru(phen)_3^{2+}$ | strong | ~1605 |
| $Al_2(C_4BP)/5'GCCGCCAT3'/5'GCCGCCAT3'/Ru(phen)_3^{2+}$ | none | 0 |

[a] ECL emission-potential transients were measured in 0.19 M phosphate buffer, pH 7, containing 0.13 M TPrA. The potential was scanned from 0 to 1.60 V at 50 mV/s.

**Table 2.** ECL of Solutions of 43 nM Ru(phen)$_3^{2+}$ with ss-DNA and ds-DNA

| solution | Ru(phen)$_3^{2+}$ alone | ss-DNA/Ru(phen)$_3^{2+}$ [a] | ds-DNA/Ru(phen)$_3^{2+}$ [b] |
|---|---|---|---|
| conc. [NP] ($\mu$M) | 0 | 57 | 33 |
| ECL[c] | 10068 ± 23 | 10052 ± 12 | 7779 ± 268 |

[a] Ratio of mol of nucleotide phosphate [NP] of ss-DNA to Ru(phen)$_3^{2+}$ = 1.3 × 10$^4$. [b] Ratio of mol of nucleotide phosphate [NP] of ds-DNA to Ru(phen)$_3^{2+}$ = 7.7 × 10$^3$. [c] Units of photomuliplier counts per second.

for 5 min, and then slowly cooled to room-temperature, during which the $\lambda$-1 ss-DNA-Ru(bpy)$_3^{2+}$ was hybridized with the complementary strand $\lambda$-1c ss-DNA on the surface as illustrated in Figure 2c. The film with the hybridized ds-DNA was employed as the working electrode in an ECL cell as described above. An ECL emission-potential transient similar to Figure 3b was observed, and the peak emission intensity is given in Table 1. However, when $\lambda$-1 ss-DNA, rather than $\lambda$-1c ss-DNA, was immobilized on the film and the same hybridization procedure described above was followed, i.e., incubating the film in the $\lambda$-1 ss-DNA-Ru(bpy)$_3^{2+}$ solution, heating to 60 °C, and then cooling to room-temperature, no ECL emission was evident. This demonstrates that the ECL emission from the film arises from the hybridization of immobilized $\lambda$-1c ss-DNA with $\lambda$-1 ss-DNA-Ru(bpy)$_3^{2+}$ in the solution. The emission again results from the oxidation of Ru(bpy)$_3^{2+}$-tagged hybridized ss-DNA and TPrA in the solution as described by eqs 1−5. In this experiment, the $Al_2(C_4BP)$ film must be incubated in the $\lambda$-1 ss-DNA solution for a sufficiently long time (at least 4 h) to cover all of the possible Al(III) binding sites on the film with the $\lambda$-1 ss-DNA before the $Al_2(C_4BP)/\lambda$-1 ss-DNA electrode is exposed to the $\lambda$-1 ss-DNA-Ru(bpy)$_3^{2+}$. Failure to cover all of the Al$^{3+}$ adsorption sites results in emission from the film, arising from some immobilization of ss-DNA-Ru(bpy)$_3^{2+}$ on the uncovered Al$^{3+}$ sites. These experiments also provide evidence that the film is stable during heating to 60 °C. If the film were unstable, the exposed gold in the control experiment (with $\lambda$-1 ss-DNA) would adsorb some $\lambda$-1 ss-DNA-Ru(bpy)$_3^{2+}$ and produce at least a low level of ECL emission. Similarly, instability of the film with labeled ds-DNA at 60 °C would result in loss of ECL emission.

**Immobilization and Hybridization of Poly(dA) and Poly-(dT).** The aluminum phosphonate film, prepared as described above, was immersed in a 21 $\mu$M [NP] poly(dA) solution for ~4 h and incubated (gradually heated to 70 °C in a water bath, held at 70 °C for 5 min, and then slowly cooled to room-temperature) in a 38 $\mu$M [NP] poly(dT) solution to produce ds-DNA. This was then washed and immersed in a 0.24 mM Ru(phen)$_3^{2+}$ aqueous solution and, following washing, was employed as a working electrode in the TPrA-containing phosphate buffer for observation of ECL. An ECL emission-potential transient similar to that in Figure 3b was observed, and the peak emission is shown in Table 1. This demonstrates that poly(dT) in the solution hybridized with the poly(dA) on the surface to produce a ds-DNA (poly(dA)·poly(dT)) that associated with Ru(phen)$_3^{2+}$ as shown in Figure 2d.[10] However, the film of poly(dA) alone, after treatment with Ru(phen)$_3^{2+}$,

did not produce ECL emission. This is consistent with the known lack of association of Ru(phen)$_3^{2+}$ with ss-DNA.

These experiments show that Ru(phen)$_3^{2+}$ only associates with ds-DNA (poly(dA)·poly(dT)), but not with ss-DNA poly-(dA). To further study the interaction of Ru(phen)$_3^{2+}$ with ss-DNA and ds-DNA, ECL emission was measured from a 43 nM Ru(phen)$_3^{2+}$ solution, a 43 nM Ru(phen)$_3^{2+}$ solution containing 57 $\mu$M [NP] of calf thymus ss-DNA, and a 43 nM Ru(phen)$_3^{2+}$ solution containing 33 $\mu$M [NP] of calf thymus ds-DNA with a QPCR analyzer. The results given in Table 2 indicate that the ECL emission decreased after ds-DNA was added to the Ru(phen)$_3^{2+}$ solution.[13] Only negligible change in the ECL signal was seen when ss-DNA was added, further demonstrating that Ru(phen)$_3^{2+}$ interacts with ds-DNA, but not with ss-DNA.

A control experiment in which a film, after the immobilization of poly(dA), was incubated in either a 21 $\mu$M [NP] poly(dA) or a 46 $\mu$M [NP] poly(dC) solution at 70 °C for 5 min, cooled to room-temperature, and then treated with Ru(phen)$_3^{2+}$ for ~2 h produced no ECL emission. This indicates that the $Al_2(C_4$-BP)/poly(dA) electrode can distinguish a complementary strand of poly(dT) DNA from noncomplementary ones.

**Immobilization and Hybridization of eight-base ss-DNA.** A similar process as described above was used to prepare the film/eight-base ss-DNA/eight-base complementary ss-DNA/Ru-(phen)$_3^{2+}$ electrode by immersing the $Al_2(C_4BP)$ film in a solution of 5'GCCGCCAT3' ss-DNA (absorbance, A, at 260 nm of 0.065) and tris buffer for 4 h, followed by incubation in a solution of the complementary 5'ATGGCGGC3' ss-DNA (A = 0.086) for 5 min at 60 °C, gradual cooling to room-temperature for 30 min to complete the hybridization reaction, and then treatment with a 0.24 mM Ru(phen)$_3^{2+}$ solution for ~1 h. The ECL emission-potential transient from the $Al_2(C_4BP)/5'GCCGCCAT3'/5'ATGGCGGC3'/Ru(phen)_3^{2+}$ electrode, similar to that in Figure 3b, was measured in the TPrA-containing phosphate buffer with the peak emission given in Table 1. A blank control experiment was carried out by incubating the $Al_2(C_4BP)/5'GCCGCCAT3'$ film in a non-complementary 5'GCCGCCAT3' ss-DNA solution (A = 0.065) and then a 0.24 mM Ru(phen)$_3^{2+}$ solution for ~1 h. No ECL emission was observed in the TPrA-containing phosphate buffer, pH 7. Taken together, these experiments demonstrate that an eight-base ss-DNA chain can be successfully immobilized on the film and is capable of recognizing the complementary eight-base strand of DNA to produce ds-DNA, which can associate with Ru(phen)$_3^{2+}$. Such a short fragment of eight-base ds-DNA might be a useful DNA probe for identifying short sequences in DNA analysis.

## Conclusions

A substrate of Au coated with an organized aluminum phosphonate film can adsorb single-stranded and double-stranded DNA. Adsorbed ss-DNA will hybridize with complementary DNA in solution to form a ds-DNA that can be detected by electrogenerated chemiluminescence of intercalated Ru-(phen)$_3^{2+}$ or a Ru(bpy)$_3^{2+}$ label attached to the DNA.

**Supplementary Material Available:** ECL emission-potential transients for the experiments summarized in Table 1 (3 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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