

A Multichannel Microfluidic Sensor That Detects Anodic Redox Reactions Indirectly Using Anodic Electrogenerated Chemiluminescence

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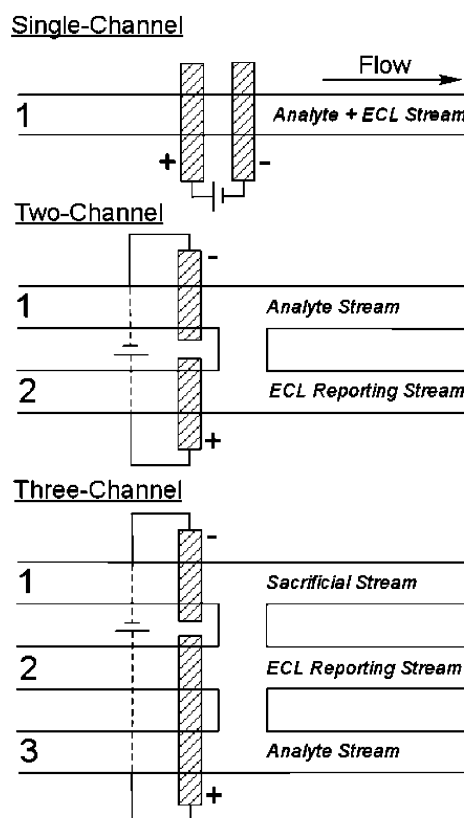
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Here, we describe a new approach for detecting redox-active targets by electrochemical oxidation and reporting their presence by electrogenerated chemiluminescence (ECL) based on electrochemical oxidation of $\text{Ru}(\text{bpy})_3^{2+}$ (bpy = 2,2'-bipyridine) and tripropylamine (TPA). This new strategy, which complements our previous reports of using ECL to signal the presence of targets undergoing electrochemical reduction, takes advantage of many of the attractive attributes of microfluidic-based electrochemical cells. These attributes include close proximity of multiple flow channels and electrodes, ability to move reagents through channels under laminar flow conditions, and the capacity to precisely place device components relative to one another using photolithography. Specifically, the microfluidic electrochemical sensor described here consists of three channels. The analyte and ECL reporting cocktail flow through separate channels, but they share a common anode. The cathode resides in a channel containing a sacrificial reductant. In this configuration, the target analyte competes with $\text{Ru}(\text{bpy})_3^{2+}$ and TPA to provide electrons for the reductant. Accordingly, in this competitive assay approach, the presence of the analyte is signaled as a lowering of the ECL intensity. In this report, the device performance characteristics are reported, and the detection of both ferrocyanide and dopamine is demonstrated at micromolar concentrations.

Here, we report the design and performance characteristics of a three-channel, two-electrode microfluidic system that detects electroactive substrates and reports their presence via electrogenerated chemiluminescence (ECL). The novel aspect of this device is that it enables detection of electroactive molecules by electrochemical oxidation and then reports their presence using electrooxidation of $\text{Ru}(\text{bpy})_3^{2+}$ (bpy = 2,2'-bipyridine) and tripropylamine (TPA); that is, both the detection and reporting reactions are based on chemically separate, but electrically correlated, electrochemical oxidation reactions.

The findings reported here complement our recent reports of using microfluidic systems for detecting electroactive molecules capable of undergoing electrochemical reduction and reporting their presence with a well-established ECL reaction cascade

Scheme 1



involving the oxidation of $\text{Ru}(\text{bpy})_3^{2+}$ and TPA.^{1,2} Specifically, as shown at the top of Scheme 1, we have previously shown that a one-channel microfluidic device, incorporating either one or two electrodes, is able to detect electrochemical processes at the cathode and report them via light emission at the anode.¹ Likewise, we have also demonstrated that a two-electrode, two-channel microfluidic device (middle of Scheme 1) can be used in the same manner, but now with complete chemical separation of the detection and reporting functions.² Both of these methods, as well as the new strategy reported here, rely on charge balance between the anode and cathode. That is, the current at the cathode must equal the current at the anode, and therefore, there is a cor-

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response between the number of electrons consumed at the cathode and the ECL photon flux at the anode.

A significant limitation of the one- and two-channel methods is that they permit detection only of targets that can be reduced. The obvious solution to this problem is to use an ECL cascade that is initiated by electrochemical reduction.³ However, there are some practical aspects of this approach that make it difficult to achieve within simple electrochemical microfluidic systems fabricated using ITO electrodes and channels formed by PDMS molds and glass slides. The approach reported here circumvents these problems.

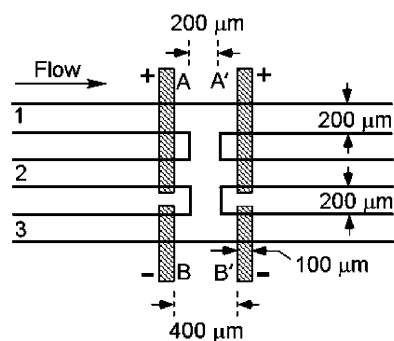
With reference to the three-channel configuration shown at the bottom of Scheme 1, our new approach to ECL reporting works as follows: Channel 1 houses the cathode and a flowing solution of a sacrificial electroactive molecule, $\text{Ru}(\text{NH}_3)_6^{3+}$ in the experiments reported here, that can be easily reduced. A solution containing $\text{Ru}(\text{bpy})_3^{2+}$ and TPA flows in channel 2, and the (oxidizable) analyte of interest is present in channel 3. Both of these channels share a common anode. When a sufficiently large potential is applied between the cathode and anode, $\text{Ru}(\text{NH}_3)_6^{3+}$ is reduced to $\text{Ru}(\text{NH}_3)_6^{2+}$, while $\text{Ru}(\text{bpy})_3^{2+}$ and TPA are oxidized. In the absence of a redox-active analyte in channel 3, maximum light emission is observed at the anode. However, when an oxidizable analyte is present in channel 3, it competes with the ECL cocktail (1 mM $\text{Ru}(\text{bpy})_3^{2+}$ and 5 mM TPA dissolved in pH 7.0 phosphate buffer) in channel 2 to provide electrons for the cathodic reduction in channel 1. Accordingly, the light intensity observed from the electrode in channel 2 will decrease, indicating the presence of the target analyte. Thus, this three-channel, two-electrode approach provides a means for detecting analytes that undergo anodic oxidation and for reporting their presence via an anodic ECL reaction that is fully compatible with the PDMS monolith and the ITO electrodes. Notice also that this three-channel design can be easily configured to detect reducible analytes. Specifically, if only buffer is present in channel 3 and the analyte is present in channel 1, then the device is essentially identical to the two-channel device shown in the middle of Scheme 1.

Electrogenerated chemiluminescence (ECL) is used as a detection method in analytical chemistry because of its intrinsic high sensitivity, low background noise, and straightforward implementation.^{4–7} Additionally, spatial and temporal control of ECL signals is intrinsic to the method.⁶ However, prior to our recent reports,^{1,2} the use of ECL for analytical detection was limited to reactions that directly involved the lumiphore (usually $\text{Ru}(\text{bpy})_3^{2+}$) or a co-reactant, such as TPA.^{8–11} Our contribution to this field has been the introduction of an approach that permits the analyte to be completely chemically decoupled from the ECL reaction cascade.

EXPERIMENTAL SECTION

Chemicals. Hexaamineruthenium chloride ($\text{Ru}(\text{NH}_3)_6\text{Cl}_3$, 99%) and $\text{Ru}(\text{bpy})_3\text{Cl}_2 \cdot 6\text{H}_2\text{O}$ (bpy = 2,2'-bipyridine) (minimum 98%) were

Scheme 2



purchased from Strem Chemicals (Newburyport, MA). Tripropylamine (TPA) (99+%), potassium ferrocyanide (II) trihydrate ($\text{Fe}(\text{CN})_6^{4-}$, 99%), and 3-hydroxytyramine hydrochloride (dopamine, 98%) were obtained from the Aldrich Chemical Co. (Milwaukee, WI). Deionized 18 M Ω -cm water (Milli-Q reagent water system, Millipore, Bedford, MA) was used to prepare all aqueous solutions. Freshly prepared 0.1 M phosphate buffer (pH 7.0) was used as the supporting electrolyte for both cyclic voltammetric and ECL measurements.

Device Fabrication. Methods used to fabricate the indium tin oxide (ITO) microelectrodes and the microfluidic devices have been reported previously.² Briefly, ITO patterning was carried out by first covering the ITO-coated glass slides with a layer of photoresist and then lithographically transferring the features on a film mask from the photoresist layer to the ITO layer using a chemical etching step.

Microfluidic devices were fabricated by a published method using poly(dimethylsiloxane) (PDMS, Dow Corning Sylgard Silicone Elastomer-184, Krayden, Inc.) molds.¹² The layout and dimensions of the device used in this work are shown in Scheme 2. PDMS molds were aligned over the patterned ITO-coated glass slides with the aid of an x,y,z micropositioner (462 series, Newport Co., Irvine, CA) and a motion controller (model 861, Newport) under a microscope having a 10 \times lens (Optiphot, Nikon). A syringe pump (PHD 2000, Harvard Apparatus, Holliston, MA) was used to deliver fluids to the channels. The potential biases applied between the two ITO microelectrodes were generated by a dc power supply (potential range, 0–25 V; model E3620A, Hewlett-Packard). Electrical contacts were made to the ITO electrodes using Ag paste (Epo-tek, Epoxy Technology, Billerica, MA).

Electrochemical Measurements. Conventional three-electrode electrochemical experiments were carried out using a Pine AFRDE4 bipotentiostat (Grove City, PA) and a Kipp and Zonen XYY' chart recorder (Bohemia, NY). Working electrodes were ITO slides (Delta Technologies, Ltd., Stillwater, MN) having an exposed area of ~ 0.45 cm², the counter electrode was a Pt wire, and the reference electrode was Ag/AgCl (3 M NaCl) (Bio-analytical Systems, West Lafayette, IN). Current measurement between the two electrodes of the microfluidic device was performed using an electrochemical workstation (model 660, CH Instruments, Austin, TX).

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ECL Measurements. The ECL signal was collected directly underneath the ITO electrode in channel 2 by means of a photomultiplier tube. This ultrahigh-sensitivity photon-counting module consisted of a channel photomultiplier, a high-voltage power supply, a discrimination amplifier, and a pulse shaper (MP 900, Perkin-Elmer). Photons generated inside the microchannel were transported to the photon-counting module through an optical fiber having a core diameter of 600 μm (M21L01, ThorLabs Inc., Newton, NJ). The TTL signal generated by the photon-counting module was then analyzed and interpreted by a multi-channel scaler (MCS) based on a PCI-bus plug-in card preinstalled on a PC.

RESULTS AND DISCUSSION

Microfluidic Device Fabrication and Characterization.

Scheme 2 illustrates the essential components of the microfluidic electrochemical system used in this study. The channels are contained within a PDMS mold bonded to a glass slide. The fluidics consist of three parallel main channels connected by a short crossover channel. All of the channels have the same width and height, 200 and 26 μm , respectively. The crossover channel has the important function of providing electrical and pressure contact between the fluids in the three main channels. Two pairs of ITO electrodes were microfabricated on the glass substrate and configured perpendicularly to the main fluidic channels. One pair of electrodes spans two adjacent channels, and one pair is confined within a single channel. Thus, each channel contains two electrodes, each having an area of $2 \times 10^4 \mu\text{m}^2$. Only one pair of electrodes, either A and B or A' and B' (Scheme 2), is used for a particular electrochemical experiment. Incorporation of two pairs of electrodes simply provides the versatility of flowing the electrolyte solution through the main channels in either direction. It will become apparent that the electrodes spanning two channels are the key elements in this system.

Figure 1a is an optical micrograph of the device illustrated in Scheme 2. The electrodes are highlighted by dashed white lines because of the poor optical contrast between ITO and glass. For electrochemical experiments, it is important that the fluids in the three channels not mix until after they encounter the active electrodes. To confirm that mixing of fluids does not occur upstream of the crossover channel, a fluorescent dye solution, 2 mM fluorescein in aqueous 0.1 M phosphate buffer (pH 6.9), was pumped through the two side channels (channels 1 and 3) at 1 $\mu\text{L}/\text{min}$, and buffer only was pumped through the middle channel (channel 2) at the same flow rate. The flow direction was from left to right. The flow profile of the three streams is shown in Figure 1b, which is a fluorescence micrograph corresponding to the optical micrograph in Figure 1a. Clearly, there is no detectable mixing of the solutions upstream of the crossover channel. Accordingly, under this flow condition, each of the three electrodes will be exposed to only the fluid in its channel. From an experimental perspective, this microchannel design makes it possible to conveniently change the analyte solution in channel 3 without disturbing flow and electrochemical processes in the other two channels.

Indirect Sensing Approach. There are many indirect detection methods used in analytical chemistry.^{13,14} Oftentimes these

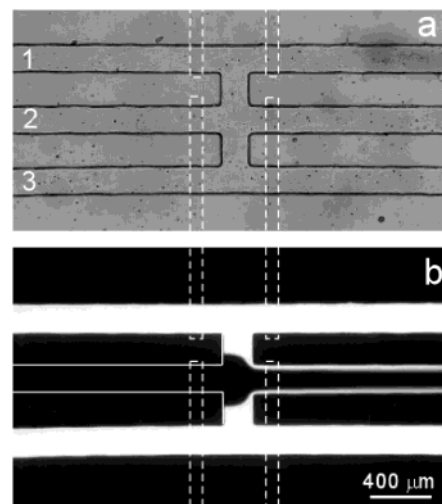


Figure 1. (a) Optical micrograph of the three-channel microfluidic device used in this study. The dashed white lines highlight the shape and positions of the microfabricated ITO electrodes. (b) Fluorescence micrograph showing the flow characteristics of the device in (a). The solid white lines highlight the middle channel. Fluorescein solution (2 mM) in 0.1 M phosphate buffer (pH 6.9) was pumped through the top and bottom channels, and buffer only was pumped through the middle channel. In all cases, the flow was from left to right at 1 $\mu\text{L}/\text{min}$. Note that there is no mixing of the fluids until they pass the first pair of ITO electrodes.

methods are designed to sense the presence of an untagged analyte in the presence of a tagged competitor. That is, the presence of the analyte is inferred from a decrease in the signal arising from the presence of the competitor. For example, in a competitive ELISA assay, the concentration of an analyte can be derived from observation of competitive binding of a tracer compound and the analyte to the antibody.¹⁵ Many other successful indirect detection methods based on fluorescence,¹⁶ conductivity,¹⁷ and absorption,¹⁸ have been reported for analytes ranging from DNA¹⁹ to chemical warfare agents.²⁰

The approach to indirect sensing reported here is carried out by configuring the microfluidic device as shown at the bottom of Scheme 1. A sacrificial stream containing $\text{Ru}(\text{NH}_3)_6^{3+}$ in phosphate buffer is pumped into channel 1. The ECL cocktail ($\text{Ru}(\text{bpy})_3^{2+}$, TPA, and phosphate buffer) flows through channel 2. Channel 3 is reserved for a buffer solution containing the analyte to be detected or a buffer-only solution. The buffer solutions in the three main channels were delivered at the same velocity to prevent potentially problematic backflow. After stable flow conditions were established, a potential of 1.60 V was applied between the two electrodes. As we have reported previously,¹ 1.60 V is sufficient to initiate reduction of $\text{Ru}(\text{NH}_3)_6^{3+}$ and oxidation of $\text{Ru}(\text{bpy})_3^{2+}/\text{TPA}$, and thus, to initiate ECL, but it is insufficient for appreciable

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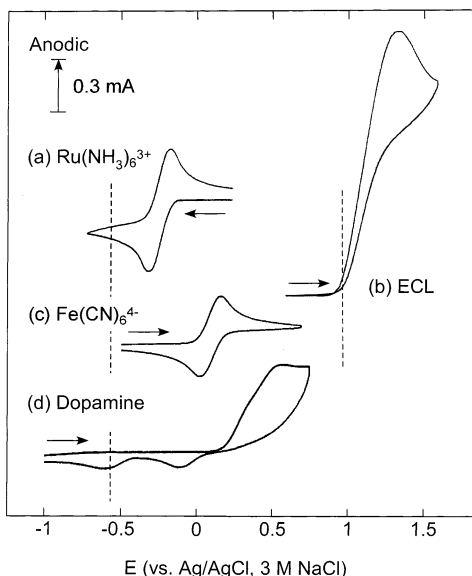


Figure 2. Cyclic voltammograms obtained using a conventional three-electrode cell. All solutions were prepared using aqueous 0.1 M phosphate buffer (pH 6.9 in (a) and (b), pH 7.0 in (c) and (d)) as the supporting electrolyte. (a) 5.0 mM $\text{Ru}(\text{NH}_3)_6\text{Cl}_3$, (b) 5.0 mM $\text{Ru}(\text{bpy})_3\text{Cl}_2$ and 25.0 mM TPA, (c) 5.0 mM $\text{K}_4\text{Fe}(\text{CN})_6$, (d) 5.0 mM Dopamine. The ITO working electrode had an area of 0.45 cm^2 , and the scan rate was 100 mV/s . The arrows indicate the initial direction of the voltammetric scan. Anodic currents are up, as indicated by the scale bar.

oxidation of the solvent or electrolyte. Accordingly, when only buffer solution is introduced to channel 3, the fraction of the anode in that channel does not contribute to the current. Under these conditions, maximum ECL output is observed in channel 2.

When an analyte that can be oxidized at a potential less than or equal to that of $\text{Ru}(\text{bpy})_3^{2+}/\text{TPA}$ is introduced into channel 3, the current demand at the cathode ($\text{Ru}(\text{NH}_3)_6^{3+}$ reduction) will then be balanced by oxidations in both channels 2 and 3 (bottom frame of Scheme 1). This results in a lower current density at the fraction of the anode in channel 2 and, therefore, a decrease in ECL intensity. The key point is that then the analyte provides some of the electrons required by the sacrificial electron acceptor in channel 1. This is the basis for indirect detection of the analyte.

Figure 2 shows the cyclic voltammetry of the redox molecules used in this study obtained using a conventional three-electrode electrochemical cell. The voltammetry of $\text{Ru}(\text{NH}_3)_6^{3+}$ ($E_{p,c} = -0.31 \text{ V}$) is shown in voltammogram a. Voltammograms b, c, and d show the voltammetry of the ECL cocktail (peak anodic current, $E_{p,a} = 1.35 \text{ V}$), $\text{Fe}(\text{CN})_6^{4-}$ ($E_{p,a} = 0.17 \text{ V}$), and dopamine ($E_{p,a} = 0.59 \text{ V}$), respectively. It is clear from these $E_{p,a}$ values that if either $\text{Fe}(\text{CN})_6^{4-}$ or dopamine is present in channel 3 of the microfluidic electrochemical cell (bottom of Scheme 1), it will compete with oxidation of $\text{Ru}(\text{bpy})_3^{2+}/\text{TPA}$ (in channel 2) when $\text{Ru}(\text{NH}_3)_6^{3+}$ is present in channel 1 and a 1.60 V bias is applied between two electrodes. Indeed, the overpotential at the anode should be sufficient to reduce these two analytes at the mass-transfer-limited rate.

We next examined the distribution of current between the two halves of the anode before and after the introduction of an analyte ($\text{Fe}(\text{CN})_6^{4-}$). This was accomplished by simultaneously monitoring the total current and the ECL intensity arising from the electrode

situated in channel 2. Control experiments indicate that the background current is 2.1 nA when channel 1 contains 1.0 mM $\text{Ru}(\text{NH}_3)_6^{3+}$ and channels 2 and 3 contain 0.1 M pH 7.0 phosphate buffer. When the buffer solution in channel 2 is changed to the ECL cocktail (1.0 mM $\text{Ru}(\text{bpy})_3^{2+} + 5.0 \text{ mM}$ TPA) the resulting current is 160 nA . This 158 nA increase in current results from electrochemical coupling of the cathodic and anodic reactions occurring in channels 1 and 2, respectively.

Next, the analyte, either 0.50 mM or 1.0 mM $\text{Fe}(\text{CN})_6^{4-}$ (in the same buffer), was introduced into channel 3. For 0.50 mM $\text{Fe}(\text{CN})_6^{4-}$, the ECL intensity dropped by 42% relative to the case when buffer only was present in channel 3, and the current remained unchanged. The presence of flowing 1.0 mM $\text{Fe}(\text{CN})_6^{4-}$ in channel 3 resulted in a 96% decrease in the ECL signal and a 3% increase in the total current. These important results indicate that the introduction of the analyte into channel 3 does not induce a significant increase in the rate of $\text{Ru}(\text{NH}_3)_6^{3+}$ reduction in channel 1. In other words, there is a redistribution of the current between the two halves of the anode when the analyte is introduced, but little increase in the total current. This means that, at least under these conditions, the critical implicit assumption that the analyte directly competes for a fixed number of electrons with the ECL cocktail is correct. Accordingly, there is a direct correspondence between the ECL intensity and the concentration of analyte in channel 3.

Optimization of Electrochemical Sensing Conditions.

In this section, we address optimization of the concentrations of the sacrificial electron acceptor, the components of the ECL stream, and the analyte. In the first set of experiments, 10.0 mM $\text{Ru}(\text{NH}_3)_6^{3+}$ in 0.1 M phosphate buffer (pH 7.0) was used as the sacrificial electron acceptor, and the concentration of $\text{Ru}(\text{bpy})_3^{2+}$ was maintained at 0.010 , 0.10 , or 1.0 mM while keeping the ratio of TPA to $\text{Ru}(\text{bpy})_3^{2+}$ constant at 5.0. Only buffer was present in the analyte stream (channel 3) for these initial experiments. Under these conditions, 0.010 mM is the lowest concentration of $\text{Ru}(\text{bpy})_3^{2+}$ that can be detected with a signal-to-noise (S/N) ratio of 2. As the $\text{Ru}(\text{bpy})_3^{2+}$ concentration was increased, the measured ECL intensity also increased.³ This finding is consistent with the expectation that current in a two-electrode cell is limited by the redox species present at the lowest concentration,²¹ in this case, the concentrations of $\text{Ru}(\text{bpy})_3^{2+}$ and TPA.

After ensuring that these control experiments performed satisfactorily, an analyte, $\text{Fe}(\text{CN})_6^{4-}$, was introduced into channel 3 of the microfluidic device, and the variation of the ECL intensity was recorded as a function of its concentration over the range $1 \mu\text{M}$ to 1.0 mM . These experiments were carried out with the $\text{Ru}(\text{NH}_3)_6^{3+}$ and $\text{Ru}(\text{bpy})_3^{2+}$ concentrations held constant at 10.0 mM and 1.0 mM , respectively. Under these condition, there was no significant change in the ECL intensity, as compared to the buffer-only case. This result is a consequence of the large excess concentration of sacrificial $\text{Ru}(\text{NH}_3)_6^{3+}$, as compared to $\text{Fe}(\text{CN})_6^{4-}$. That is, when the sacrificial electron acceptor is in large excess, it is able to supply all the current necessary to drive oxidation of both $\text{Fe}(\text{CN})_6^{4-}$ and $\text{Ru}(\text{bpy})_3^{2+}/\text{TPA}$ at their mass-transfer-limited rate.

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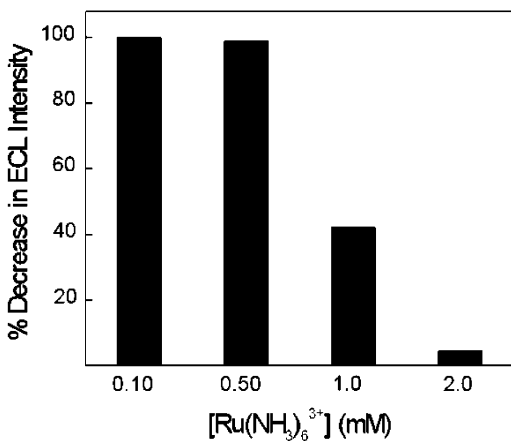


Figure 3. Percentage decrease in the initial (buffer only in channel 3, Scheme 2) ECL intensity after 0.50 mM $\text{Fe}(\text{CN})_6^{4-}$ is introduced into channel 3. The ECL cocktail in channel 2 contained 1.0 mM $\text{Ru}(\text{bpy})_3^{2+}$ /5.0 mM TPA. The $\text{Ru}(\text{NH}_3)_6^{3+}$ concentration in channel 1 varied from 0.10 to 2.0 mM. The potential bias applied between the two electrodes was 1.60 V in all cases.

In another set of experiments, we held the concentrations of $\text{Ru}(\text{bpy})_3^{2+}$ and $\text{Fe}(\text{CN})_6^{4-}$ constant at 1.0 and 0.50 mM, respectively, and recorded the ECL signal using concentrations of the sacrificial electron acceptor ($\text{Ru}(\text{NH}_3)_6^{3+}$) between 0.10 and 2.0 mM. The results of these experiments are given in Figure 3. When the concentration of $\text{Ru}(\text{NH}_3)_6^{3+}$ is relatively low (0.10 or 0.50 mM), the current necessary to reduce it is nearly completely supplied by the oxidation of $\text{Fe}(\text{CN})_6^{4-}$, which leads to a nearly 100% decrease in the initial ECL signal. Therefore, under these conditions, the concentration of the analyte cannot be accurately indicated by the decrease in the ECL response. At relatively high $\text{Ru}(\text{NH}_3)_6^{3+}$ concentration (2.0 mM), the situation is similar to that discussed in the previous paragraph. That is, the concentration of sacrificial $\text{Ru}(\text{NH}_3)_6^{3+}$ is sufficiently high that mass-transfer-limited oxidation of both $\text{Ru}(\text{bpy})_3^{2+}$ and $\text{Fe}(\text{CN})_6^{4-}$ can be accommodated with little decrease of ECL signal. When the concentration of $\text{Ru}(\text{NH}_3)_6^{3+}$ is equal to 1.0 mM, however, then the decrease in ECL intensity (~40%) reflects the presence of the analyte. We then conclude that the highest sensitivity of this indirect sensing approach occurs when the concentrations of the redox-active species present in all three channels are roughly comparable to one-another.

Detection of Target Analytes by Anodic Oxidation. A central conclusion of the last section is that the concentrations of the sacrificial solution and the ECL solution should be closely matched to observe indirect sensing effects with maximum sensitivity. Accordingly, we performed the following sensing experiments using 1.0 mM $\text{Ru}(\text{NH}_3)_6^{3+}$ in channel 1 and 1.0 mM $\text{Ru}(\text{bpy})_3^{2+}$ /5.0 mM TPA in channel 2. Two targets were independently analyzed in channel 3: ferrocyanide, which is a well-understood electrochemical probe that undergoes a nearly reversible electron transfer on ITO electrodes (voltammogram c in Figure 2), and dopamine, which is a neurotransmitter that behaves irreversibly on ITO (voltammogram d in Figure 2). The measured $E_{p,a}$ values for these two compounds are 0.17 and 0.59 V, respectively. Comparison of these values with the $E_{p,a}$ for the ECL cascade reaction, 1.35 V, indicates that the two analytes are more

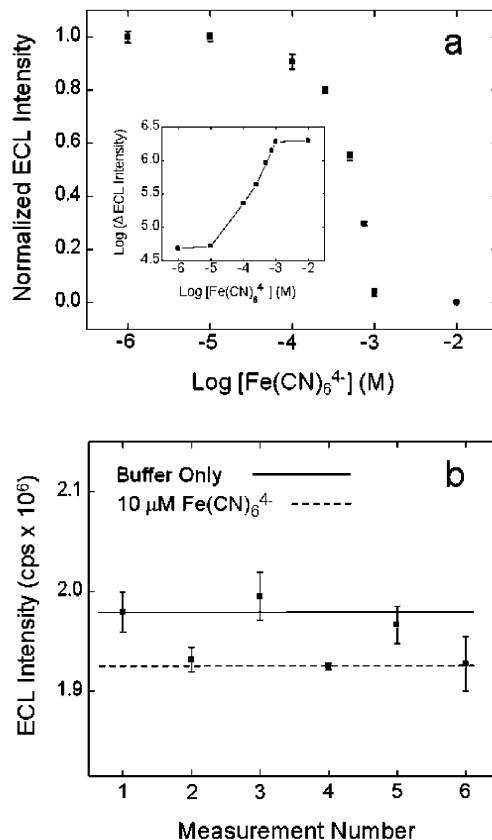


Figure 4. Indirect sensing of $\text{Fe}(\text{CN})_6^{4-}$. The sacrificial stream (channel 1, Scheme 2) and the ECL reporting stream (channel 2) contained 1.0 mM $\text{Ru}(\text{NH}_3)_6^{3+}$ and 1.0 mM $\text{Ru}(\text{bpy})_3^{2+}$ /5.0 mM TPA, respectively, dissolved in 0.1 M, pH 7.0 phosphate buffer. The flow rate of all solutions was 1 $\mu\text{L}/\text{min}$. $\text{Fe}(\text{CN})_6^{4-}$ ranging in concentration from 1 μM to 10.0 mM was present in channel 3. (a) Normalized ECL intensity vs the logarithm of the $\text{Fe}(\text{CN})_6^{4-}$ concentration. The potential bias applied between the two electrodes was 1.60 V in all cases. The inset is a log-log plot of the decrease in ECL intensity (subtracted from the initial ECL signal observed in the absence of $\text{Fe}(\text{CN})_6^{4-}$) vs the $\text{Fe}(\text{CN})_6^{4-}$ concentration. The ECL intensity was recorded as counts per second, cps. (b) ECL intensity for six consecutive measurements made by alternatively introducing only buffer or buffer plus 10.0 μM $\text{Fe}(\text{CN})_6^{4-}$ into channel 3.

easily oxidized than the ECL cocktail. As a result, both analytes will compete with the ECL reaction to donate electrons to $\text{Ru}(\text{NH}_3)_6^{3+}$.

Results corresponding to indirect ECL sensing of $\text{Fe}(\text{CN})_6^{4-}$ are shown in Figure 4. Figure 4a is a plot of normalized ECL intensity as a function of the logarithm of the $\text{Fe}(\text{CN})_6^{4-}$ concentration flowing in channel 3 (Scheme 2). The potential bias applied between the anodes and cathode was kept constant at 1.60 V throughout these measurements. The most sensitive part of this plot occurs when the concentration of $\text{Fe}(\text{CN})_6^{4-}$ is in the range 0.10 to 1.0 mM. Recall that the concentrations of $\text{Ru}(\text{NH}_3)_6^{3+}$ and $\text{Ru}(\text{bpy})_3^{2+}$ are both set to 1.0 mM in these experiments, so this result confirms our previous finding that analytes present at roughly the same concentration as the sacrificial acceptor and lumiphore are most easily sensed. As shown in Figure 4b, reproducible decreases in ECL intensity are observed for $\text{Fe}(\text{CN})_6^{4-}$ concentrations as low as 10 μM under these conditions.

When the concentrations of $\text{Ru}(\text{NH}_3)_6^{3+}$, $\text{Ru}(\text{bpy})_3^{2+}$, and $\text{Fe}(\text{CN})_6^{4-}$ were all equal to 1.0 mM, <5% of the initial ECL

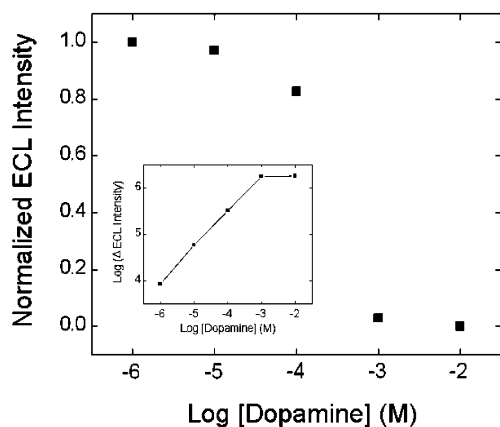


Figure 5. Plot of normalized ECL intensity vs the logarithm of dopamine concentration. The solutions in channels 1 (Scheme 2) and 2 were 1.0 mM $\text{Ru}(\text{NH}_3)_6^{3+}$ and 1.0 mM $\text{Ru}(\text{bpy})_3^{2+}$ /5.0 mM TPA, respectively, in 0.1 M, pH 7.0 phosphate buffer. The flow rate of the all of the solutions was 1 $\mu\text{L}/\text{min}$. Dopamine, present at concentrations between 1 μM and 10.0 mM, was present in channel 3. The potential bias applied between the two electrodes was 1.60 V in all cases. The inset is a log–log plot of the decrease in ECL intensity (subtracted from the initial ECL signal observed in the absence of dopamine) vs the dopamine concentration.

intensity ($\text{Fe}(\text{CN})_6^{4-}$ absent) appeared. This is probably a consequence of the much smaller potential bias required to turn on electrochemical coupling between $\text{Ru}(\text{NH}_3)_6^{3+}$ and $\text{Fe}(\text{CN})_6^{4-}$ ($|E_{\text{p,a}}(\text{Fe}(\text{CN})_6^{4-}) - E_{\text{p,c}}(\text{Ru}(\text{NH}_3)_6^{3+})| = 0.48 \text{ V}$), compared to that of $\text{Ru}(\text{NH}_3)_6^{3+}$ and the ECL cocktail ($|E_{\text{p,a}}(\text{ECL}) - E_{\text{p,c}}(\text{Ru}(\text{NH}_3)_6^{3+})| = 1.66 \text{ V}$). Thus, although the two oxidation reactions occur at the same anode, the oxidation of $\text{Fe}(\text{CN})_6^{4-}$ is driven at its mass-transfer-limit rate, whereas the oxidation of $\text{Ru}(\text{bpy})_3^{2+}$ and TPA occur at the foot of the voltammetric wave. In this case, the relative potentials of the anode and cathode reactions must be close to the dashed lines shown in Figure 2. Under these same conditions, but with the concentration of $\text{Fe}(\text{CN})_6^{4-}$ 10 times higher than that of $\text{Ru}(\text{bpy})_3^{2+}$, no detectable ECL is observed. The inset of Figure 4a is a plot of the logarithm of the decrease in ECL intensity (from its value in the absence of $\text{Fe}(\text{CN})_6^{4-}$) as a function of the logarithm of the $\text{Fe}(\text{CN})_6^{4-}$ concentration. This nearly linear calibration curve provides a direct link between ECL intensity and the analyte concentration.

Trends similar to those described for $\text{Fe}(\text{CN})_6^{4-}$ were also found for dopamine (Figure 5). For example, the most sensitive part of the plot of normalized ECL intensity vs the logarithm of the dopamine concentration is in the range 0.10–1.0 mM dopam-

ine. Likewise, the inset shows that the log–log calibration plot is nearly linear over 3 orders of magnitude of dopamine concentration. Thus, we believe the indirect sensing strategy presented here can be generally used for the detection of redox compounds that can undergo oxidation. The only requirement for this system to work is that the analytes must be electroactive at an ITO electrode.

SUMMARY AND CONCLUSIONS

In two previous reports^{1,2} we showed that it was possible to chemically decouple electrochemical detection and ECL-based reporting functions in a microfluidic sensor. Specifically, we demonstrated that the presence of targets that could undergo electrochemical reduction could be sensed and their presence reported by $\text{Ru}(\text{bpy})_3^{2+}$ -based ECL. However, because of the constraints placed on the electrodes and the materials used to fabricate the microfluidic device by the ECL reporter reaction cascade, it was inconvenient to detect targets that could be electrochemically oxidized. Here, we have addressed this issue by developing a simple, competitive assay in which the analyte and $\text{Ru}(\text{bpy})_3^{2+}$ compete to provide electrons for a sacrificial reagent. We found that this approach works best when the ECL and sacrificial reagents are present at about the same concentration as the analyte to be sensed. However, it is unlikely that the ultimate concentration sensitivity of this indirect sensing strategy will be as low as the direct ECL reporting strategy we reported previously.

Perhaps the most significant aspect of these results is that they demonstrate the flexibility of using microfluidics for implementing electrochemical sensing schemes. In this regard, microfluidic devices have the following advantages when compared to traditional multimilliliter electrochemical cells: very small cell volume and overall device size, close proximity of multiple channels and electrodes, ease of moving reagents through channels under laminar flow conditions, simple integration of additional functions onto the microfluidic device, and ability to precisely place device components relative to one-another using photolithography.^{22–25}

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