Combining Micromachining and Molecular Self-Assembly To Fabricate Microelectrodes

Nicholas L. Abbott*
Department of Chemical Engineering and Materials Science, University of California, Davis, California 95616

Debra R. Rolison*
Code 6170, Surface Chemistry Branch, Naval Research Laboratory, Washington, D.C. 20375-5342

George M. Whitesides'
Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138

Received February 16, 1994. In Final Form: May 9, 1994

This paper describes three types of gold electrodes, each with at least one spatial dimension as small as 1 μm, fabricated using combinations of micromachining and molecular self-assembly. (i) Large-area (>0.1 mm²) arrays of band-type electrodes were formed by machining micrometer-wide grooves of bare gold into gold film covered with a ~23-A-thick, electrically insulating, self-assembled monolayer (SAM) of CH₃(CH₂)₁₃S. (ii) Micrometer-wide, centimeter-long, 100-nm-thick gold electrodes supported on glass were fabricated by patterning a gold film with monolayers of CH₃(CH₂)₁₃S and HO(CH₂)₁₃S, and selectively etching the regions of gold covered with HO(CH₂)₁₃S using an aqueous solution of CN⁻ saturated with O₂. (iii) Two microelectrodes, each with electrochemically active areas as small as 100 nm × 1 μm and separated from each other by a distance of 1 μm, were formed by machining a 1-μm-wide gap into a supported gold electrode covered with an electrically insulating SAM of CH₃(CH₂)₁₃S, exposed gold on either side of the machined gap formed the electrochemically active surfaces of the microelectrode pair.

Introduction

Microelectrodes are electrodes with at least one micrometer-scale dimension.¹ ² These small electrodes can be used to investigate electrochemistry with a spatial resolution on the order of the size of the electrode. Because microelectrodes have small capacitances (due to their small area) and because the flux of redox-active species to small electrodes is high (due to nonplanar diffusion), these electrodes can also be used to make electrochemical measurements on fast processes.² These characteristics, and others, have lead to applications of microelectrodes in electrophysiology,³ the measurement of kinetics of heterogeneous chemical reactions,² ⁵ and fabrication of electroanalytical devices.⁶ Only one dimension of an electrode needs to be small for fast response times (<milliseconds). Arrays of band-type microelectrodes—electrodes with micrometer-scale lateral dimensions and millimeter-scale longitudinal dimensions—combine relatively large electrode areas (>1000 μm²) with millisecond response times.⁷ Electrodes with both lateral and longitudinal dimensions in the micrometer range can measure, in principle, the presence of redox-active analytes in volumes as small as ~1 fL (1 pm³). Self-assembled monolayers (SAMs) of long-chain alkanethiolates on gold have been used extensively to design and synthesize organic surfaces with well-defined chemical and physical properties.⁸ ²³ In this work we used SAMs for two reasons. First, SAMs⁴ of CH₃(CH₂)₁₃S

---


effectively block the transfer of electrons between a film of gold and a contacting aqueous solution of redox-active molecules.\textsuperscript{14-23} Regions of bare\textsuperscript{26} gold micromachined\textsuperscript{26} into these surfaces behave as microelectrodes.\textsuperscript{22} Second, SAMs of CH\textsubscript{3}/CH\textsubscript{2}/S protect films of gold from wet chemical etching by solutions of CN\textsuperscript{-} saturated with O\textsubscript{2}.\textsuperscript{6} SAMs of HO/CH\textsubscript{2}/S do not, however, form a protective barrier to this etchant. Selective etching of films of gold patterned with micrometer-scale regions of monolayers of CH\textsubscript{3}/CH\textsubscript{2}/S and HO/CH\textsubscript{2}/S forms microwires of gold supported on glass. These supported microwires function as microelectrodes.

The procedures we report for preparing microelectrodes of gold are general, do not require photolithography, and can be followed in any wet chemical laboratory. The bare gold surfaces of the electrodes can be functionalized with redox-active species and redox catalysts, making this method of preparation well-suited for the rapid prototyping of sensors and devices. Because the sequence of micromachining and selective deposition of SAMs on machined regions of gold can be performed repeatedly on the same sample, the procedures reported in this paper can be used to form surfaces with multiple, spatially-separated, microscopic areas each functionalized with a different surface-confined species. This capability, we believe, will make this procedure a useful one for fabricating structures with more than one surface-confined species.

### Results and Discussion

#### Fabrication of Microelectrodes

The fabrication of three types of microelectrodes is described below; the procedures are detailed in the Experimental Section. All potentials are referenced to a saturated calomel electrode (SCE), unless indicated otherwise.

**A. "Groove" Electrodes.** "Groove" electrodes were formed by using the tip of a surgical scalpel blade to machine micrometer-wide grooves into a film of gold supporting a SAM of CH\textsubscript{3}/CH\textsubscript{2}/S (Figure 1). Gold exposed by machining formed the electrochemically active surface of the microelectrode. Figure 2a is a scanning electron micrograph (SEM) of an array of grooves. Arrays of grooves (10 grooves \times 10 grooves), rather than a single groove, were used to increase the magnitude of the current coming from them relative to the background currents (i.e., it improved the ratio of signal to noise). Each groove had a macroscopic length (\approx 1 cm), width of \approx 1 \mu m, and depth of \approx 0.05 \mu m (Figure 2c).\textsuperscript{9} The distance between parallel grooves was 500–1000 \mu m. Figure 2b shows an SEM of the intersection of two \approx 1-\mu m-wide grooves. Arrays of grooves with widths of \approx 8 \mu m were also prepared (Figure 2d) by machining the SAM with a larger load applied to the machine tip (see Experimental Section). Cyclic voltammetry in aqueous solutions of 0.1 M H\textsubscript{2}SO\textsubscript{4} was used to estimate the effective surface area of electrochemically active gold in the grooves. Figure 3a,\textsuperscript{15-17} Figure 3a shows a cyclic voltammogram measured using a macroscopic, polycrystalline film of gold (\approx 1 cm\textsuperscript{2}), the anodic current begins at +1.1 V and is the sum of a small peak (shoulder) at +1.15 V and a large peak at +1.26 V. The presence of two peaks, and their relative sizes and positions, is similar to the two-electron oxidation of single-crystal Au(111)\textsuperscript{15} immersed in an aqueous solution of either 0.1 M H\textsubscript{2}SO\textsubscript{4}\textsuperscript{16} or 0.05 M H\textsubscript{2}SO\textsubscript{4}.\textsuperscript{19} Because the anodic currents are probably convolved with a contribution from the oxidation of water, the areas of the anodic peaks are not reliable for estimating the effective surface area of gold on the film.\textsuperscript{15,17,18} The anodic peak at +0.87 V corresponds to the reduction of the surface layer of gold oxide to gold, and is used below to estimate the area of bare gold on the surface of the film.

The small size of the peak seen in the cyclic voltammogram of Figure 3b indicates that electrochemical oxidation of the gold film was essentially suppressed by the formation of a SAM of CH\textsubscript{3}/CH\textsubscript{2}/S on its surface. From the relative areas of the cathodic peaks in Figure 3a,b, the effective surface area of electrochemically active gold on the SAM-covered surface was estimated to be 0.01% of the surface area of the gold film before the SAM was formed (Table 1). Because applied electrical potentials in aqueous solution can disrupt sulfur–gold bonds, this estimate of the effective area of bare gold can include a contribution caused by the method of measurement and should, therefore, be considered an upper bound.\textsuperscript{19}

The potential for reduction of gold oxide on a gold film supporting a SAM of CH\textsubscript{3}/CH\textsubscript{2}/S was 50 mV more negative than that measured using the macroscopic
Figure 2. Scanning electron micrographs (SEMs) of micrometer-wide grooves of bare gold machined into a gold film covered with a SAM of CH₃(CH₂)₁₂S. (a) An array of 1-μm-wide grooves. The dark spot in the center of the image was caused by a contaminating particle on the surface of the gold film. The accelerating voltage \( (V_a) \) was 20 kV. (b) Top view of the intersection of two ~1-μm-wide grooves \( (V_a = 35 \text{ kV}) \). (c) A cross-sectional view of a micrometer-wide groove. The gold film was supported on a wafer of silicon that was fractured normal to the groove for imaging \( (V_a = 25 \text{ kV}) \). (d) The intersection of two 8-μm-wide grooves that were part of an array (not shown) \( (V_a = 35 \text{ kV}) \).

Table 1. Voltammetric Peak Potentials and Geometric Areas of Electrodes in Aqueous 0.1 M H₂SO₄ Electrolyte

<table>
<thead>
<tr>
<th>electrode</th>
<th>geometric area</th>
<th>( E_{p,a} ) (V vs SCE) for oxidation of Au</th>
<th>( E_{p,r} ) (V vs SCE) for reduction of gold oxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>bare Au</td>
<td>1 cm²</td>
<td>+1.15, +1.26</td>
<td>+0.75</td>
</tr>
<tr>
<td>Au + CH₃(CH₂)₁₂S</td>
<td>10 000 μm²</td>
<td>e</td>
<td>+0.82</td>
</tr>
<tr>
<td>array of 1-μm grooves</td>
<td>90 000 μm²</td>
<td>+1.10 (shoulder), +1.20</td>
<td>+0.87</td>
</tr>
<tr>
<td>wire</td>
<td>11 000 μm²</td>
<td>+1.17</td>
<td>+0.83</td>
</tr>
</tbody>
</table>

\( ^a \) Data obtained at a scan rate of 0.1 V/s. \( ^b \) Attempts to obtain the voltammetric behavior of gap electrodes in aqueous 0.1 M H₂SO₄ were unsuccessful. The electrodes were displaced from the supporting glass substrate during cyclic voltammetry. \( ^c \) Determined from the ratio of the area of the peak for the reduction of gold oxide at the designated electrode to that at a nominal 1 cm² bare gold electrode. \( ^d \) The area of the gold oxide reduction peak indicates a surface roughness (based on a two-electron reduction of a single-crystal Au(111) surface equal to 444 μC/cm²)² of ~2.7 for the evaporatively deposited Au film electrode. \( ^e \) A well-defined oxidation process was not seen (Figure 3a).

The oxidation of gold underlying a SAM and subsequent reduction of gold oxide so formed require the permeation of the SAM by water and ions, and because the SAM is hydrophobic and has a low dielectric constant, it is reasonable that the potentials for both redox processes

surface of gold (Figure 3 and Table 1).
The capacitance using $C = \varepsilon e d / 2x$, where $\varepsilon = 2.26$ (the value for polyethylene), $\varepsilon_a = 8.854 \times 10^{-14}$ F/cm, and $d_{nm} = 2.3 \times 10^{-7}$ cm (assuming the alkane chain is fully extended and tilted 30° from the normal). The capacitance calculated is $0.87 \mu F/cm^2$, in close agreement with the value estimated from ref 19 (see text).

The capacitance of the SAM of CH$_x$(CH$_2$)$_{15}$S on gold was estimated from Figure 3b using eq 1, where $C$ is the capacitance ($\mu F/cm^2$) and $\Delta\varphi$ is the accumulation of charge ($\mu C/cm^2$) caused by a change in electrical potential $\Delta E$ (V). For a change in potential from $-0.125$ to $+0.125$ V,

$$C = \Delta\varphi / \Delta E$$

The capacitance of the SAM of CH$_x$(CH$_2$)$_{15}$S on gold was estimated from Figure 3b using eq 1, where $C$ is the capacitance ($\mu F/cm^2$) and $\Delta\varphi$ is the accumulation of charge ($\mu C/cm^2$) caused by a change in electrical potential $\Delta E$ (V). For a change in potential from $-0.125$ to $+0.125$ V.

Figure 3. Cyclic voltammograms measured in aqueous solutions of 0.1 M H$_2$SO$_4$: (a) a macroscopic film of gold evaporated on a glass microscope slide ($\sim 1$ cm$^2$); (b) a film of gold covered with a SAM of CH$_x$(CH$_2$)$_{15}$S ($\sim 1$ cm$^2$); (c) an array of micrometer-wide grooves micromachined into a film of gold covered with a SAM of CH$_x$(CH$_2$)$_{15}$S (same sample as middle cyclic voltammogram). The potential was scanned at 100 mV/s. Note the different scales on the current axes. The capacitive currents (shaded) correspond to the reduction of surface layers of gold oxide to gold, and were used to estimate the area of electrochemically active gold on the surface of the films. The continuous and vertical lines passing through the capacitive peaks is positioned at 0.87 V.

Figure 3 shows a cyclic voltammogram measured using an array of grooves (10 rows by 10 columns) micromachined into a SAM of CH$_x$(CH$_2$)$_{15}$S. The shape and positions of the anodic peaks corresponding to the oxidation of the machine-exposed gold within the grooves have changed relative to those obtained for the evaporated film of gold (Figure 3a). This change is plausibly caused by the exposure of different facets of gold on the surface of the machined groove as compared to the evaporated surface of predominantly Au(111). The prominent anodic peak at $+1.20$ V seen in Figure 3c and the slight shoulder at $-1.1$ V are consistent with oxide formation on a Au(100) surface in 0.05 M H$_2$SO$_4$. If a (111) crystallographic plane is cut open, (100) facets should be exposed. The electrochemically active surface area of gold in the array of micromachined grooves was estimated to be 0.09% of that present on the 1 cm$^2$ of bare gold from relative areas of the cathodic peaks.

Knowing the total length of the grooves ($\sim 20$ cm), and assigning a planar band geometry to the micromachined groove, we calculated the effective width of electrochemically active gold in the micromachined grooves to be $0.5 \mu m$. This value is slightly smaller than the width of deformed gold seen in the SEM ($\sim 1$ cm; see Figure 2b). The disagreement between the widths of the grooves obtained by the two methods is not unreasonable because some fraction of the area of the deformed gold in and around the micromachined grooves is probably still covered by SAM and because the surface of the grooves can consist of less densely packed planes of Au than Au(111).

Upon scanning negative of 0 V with the grooved array, an increase in cathodic current was observed (Figure 3c), unlike the response for either bare Au (Figure 3a) or Au supporting a SAM of CH$_x$(CH$_2$)$_{15}$S (Figure 3b). Because the onset of the cathodic current observed in Figure 3c was displaced to $-0.8$ V at pH 7 (not shown), and because a comparable reduction of protons at the bare Au at these potentials does not appear to be occurring at comparable current densities, the process is giving rise to the current appearing related to a proton-dependent reduction involving remnants of a SAM in the groove. Alternatively, exposed facets of gold other than Au(111) could be mediating the process leading to the cathodic current as the kinetics for proton electroreduction are slower at Au(111) relative to the other gold surfaces.

B. “Wire” Electrodes. “Wire” electrodes were prepared using the three-step procedure shown in Figure 4. First, micrometer-wide grooves of bare gold were machined into a film of gold supporting a monolayer of HO(CH$_2$)$_{15}$S. The machined grooves of gold were covered selectively with SAMs of CH$_x$(CH$_2$)$_{15}$S. Third, the areas of the gold film covered with HO(CH$_2$)$_{15}$S were etched selectively with an aqueous solution of CN$^-$ that was saturated with O$_2$. This procedure was used to fabricate wires supported on glass with widths of $\sim 1$ mm, lengths of $\sim 1$ cm, and top surfaces covered with SAMs of CH$_x$(CH$_2$)$_{15}$S. The fabrication of wire electrodes relies on two
The area of electrochemically active gold on the supported wire (relative to bare Au) was estimated to be 11,000 \( \mu \text{m}^2 \) using cyclic voltammetry in an aqueous solution of 0.1 M H\(_2\)SO\(_4\), as described above (Table 1). Assuming the electrochemically active surface of gold to be the walls of the wire (the top of the wires is covered with a SAM of CH\(_3\)CH\(_2\)SH), we calculated the geometric area of exposed gold to be 2 \( \times \) 0.1 \( \mu \text{m} \times 13 \text{ mm} = 2600 \mu \text{m}^2 \) (0.1 \( \mu \text{m} \) corresponds to the height of the walls of the wire, and 13 mm corresponds to the length of the wire electrode). The measured electrochemically active surface area of the electrode is larger, by a factor of 4, than the calculated surface area of the walls. Possible explanations for the difference between the estimates include exposed gold on the top surface,\(^{34,35}\) of the supported wire and roughness on the edges and faces of the wire (see Figure 5) caused by the etch procedure (which would lead to greater electrochemically active area than anticipated on the basis of the calculations of geometric area).

C. "Gap" Electrodes. "Gap" microelectrodes were prepared from supported wire electrodes connected at both ends to electrical contact pads (see above description for the preparation of supported wire electrodes). First, exposed gold surfaces of the wire were covered with a SAM of CH\(_3\)CH\(_2\)SH. To form an electrically insulating SAM of CH\(_3\)CH\(_2\)SH on the walls of the wires, it was necessary to clean the wires electrochemically by cycling the potential of the wires between -0.4 and 1.4 V in an aqueous solution of 0.1 M H\(_2\)SO\(_4\) prior to immersing the wires in an ethanolic solution of CH\(_3\)CH\(_2\)SH. We attributed the necessity of the pretreatment to poisoning of the Au surface by strongly adsorbed CN, which, unlike adventitious adsorbates on Au, did not appear to be completely displaced by the thiol moiety.\(^{35}\) Second, a 1-\(\mu\)m-wide gap was machined into the wire using the tip of a surgical scalpel as the machining tool (Figure 6). The machine-exposed areas of gold on either side of the gap were used as working and counter electrodes. The geometry of the gap varied from one sample to another. Residual material (probably gold machined from the gap) was sometimes observed by scanning electron microscopy to be deposited on either side of the gap as shown in Figure 6a. Although this residual material was not always present, as seen in Figure 6b, holes that formed during the etching of the film of gold could be observed in the wire by scanning electron microscopy.

**Electrochemical Characterization of Microelectrodes.** We measured cyclic voltammograms using groove, wire, and gap electrodes in aqueous solutions of K\(_2\)Fe(CN)\(_{6}\), K\(_2\)FeCN\(_{6}\), and Ru(NH\(_2\))\(_3\)Cl\(_3\) and solutions of acetonitrile containing ferrocene. Figure 7 is a schematic illustration of the experimental setup used to measure the cyclic voltammograms of these electrodes. Details are presented in the Experimental Section. The voltammetric data obtained from these studies are summarized in Table 2.

A. Groove Electrodes. The cyclic voltammogram shown in Figure 8a was measured using a macroscopic film of gold (~0.2 cm\(^2\)) immersed in an aqueous solution of 1 mM K\(_2\)Fe(CN)\(_{6}\), 10 mM K\(_2\)HPO\(_4\) (pH 7.0), and 0.1 M NaClO\(_4\). The cathodic peak (\(E_p = 0.13 \text{ V}\)) corresponds to the reduction of Fe(CN)\(_{6}\)\(^{3-}\) to Fe(CN)\(_{6}\)\(^{4-}\). The peak-to-peak separation of the cathodic and anodic waves was ~70 mV. The cathodic peak current (\(i_{p,c}\)) was measured as a function of the scan rate (\(v\)) for scan rates between 10 and 100 mV/s: current and rate were related by the

\[ \text{rate} = \text{constant} \times \text{current} \]

The calculated geometric area of the top surface of the wire is ~1 \(\mu\text{m} \times 13 \text{ mm} = 13000 \mu\text{m}^2\).

Combining Micromachining and Molecular Self-Assembly

Figure 6. Scanning electron micrographs of gap electrodes. (a) A 1-μm-wide gap machined into a 1-μm-wide supported wire of gold. Note that, in addition to the 1-μm-wide supported wire of gold, a 0.1-μm-wide wire that runs parallel to the 1-μm-wide wire is visible in (a); the two wires are separated by ~300 nm. The residual material at the end of each electrode is probably gold that was machined from the gap. This gold may contribute to the working area of the electrode. (b) An approximately 1-μm-wide gap machined into a 1-μm-wide wire. At this magnification holes are visible in the wires. The accelerating voltage was 5 kV in both images.

The power law \( i_h \sim \nu^{0.45} \). The exponent of \( \nu \) contains information about the nature of the mass transport at the electrode. For a surface-confined, nondiffusing redox solute, an exponent of 1 is obtained. When semi-infinite (planar) diffusion controls mass transport, the exponent is 0.5 (\( i_h \) is proportional to \( \nu^{1/2} \)). When finite diffusion describes the mass transport of the electroactive solute, as has been observed for polymer-modified electrodes, an exponent between 0.5 and 1.0 is obtained. When non-planar (spherical or cylindrical) diffusion describes the mass transport (as occurs for microelectrodes), the exponent falls below 0.5. For hydrodynamic voltammetry, as at rotated disk electrodes, the limiting current is essentially independent of scan rate for the range of scan rates used here (10–100 mV/s); i.e., the exponent is zero. The exponent of 0.45 measured using a macroscopic Au electrode is close to 0.5 as expected for planar diffusion of a redox-active species to an electrode.\(^{1,2}\)

Figure 7. Schematic illustrations of the experimental apparatus for cyclic voltammetry: (a) groove and wire electrodes; (b) gap electrodes. The volume of the drop was typically 10–20 μL. A detailed description is given in the Experimental Section.

Table 2. Voltammetric Peak Potentials and Currents of Groove and Wire Electrodes in Aqueous Solutions of Fe(CN)₆³⁻ and Ferrocene in Acetonitrile

<table>
<thead>
<tr>
<th>Electrode</th>
<th>Reduction of Fe(CN)₆³⁻, pH 7, 0.1 M NaClO₄ in Water</th>
<th>Ferrocene in 0.1 M Bu₄NPF₆/CH₂CN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bare Au</td>
<td>(+0.17 \text{ mV vs SCE})</td>
<td>(+0.40 \text{ mV vs SCE})</td>
</tr>
<tr>
<td>Array of grooves</td>
<td>+0.18 \text{ mV}</td>
<td>+0.20 \text{ mV}</td>
</tr>
<tr>
<td>1 μm</td>
<td>+0.18 \text{ mV}</td>
<td>+0.18 \text{ mV}</td>
</tr>
<tr>
<td>0.1 μm</td>
<td>+0.18 \text{ mV}</td>
<td>+0.18 \text{ mV}</td>
</tr>
<tr>
<td>Wire</td>
<td>+0.18 \text{ mV}</td>
<td>+0.18 \text{ mV}</td>
</tr>
<tr>
<td>Array of grooves</td>
<td>+0.18 \text{ mV}</td>
<td>+0.18 \text{ mV}</td>
</tr>
<tr>
<td>1 μm</td>
<td>+0.18 \text{ mV}</td>
<td>+0.18 \text{ mV}</td>
</tr>
<tr>
<td>0.1 μm</td>
<td>+0.18 \text{ mV}</td>
<td>+0.18 \text{ mV}</td>
</tr>
<tr>
<td>Wire</td>
<td>+0.18 \text{ mV}</td>
<td>+0.18 \text{ mV}</td>
</tr>
</tbody>
</table>

The area of the macroscopic electrode is only known approximately (~0.2 cm). We cannot, therefore, make a quantitative comparison between experiment and calculation.\(^{3}\) The scan rate was 10 mV/s. The separation of the peaks was 60 mV.

suggests a limitation to the rate of transfer of electrons through the SAM (or defects in the SAM) to the electrode (see Figure 3b), rather than a diffusion-limited current. The current arising from heterogeneous transfer of electrons can be described by the Butler–Volmer equation,\(^{36}\)

\[ i = nF A k \left[ (c_e e^{-\alpha n F E^*}) - (c_e e^{-(1-\alpha n F E^*)}) \right] \]

where \( n \) is the moles of charge transferred per mole of

redox species arriving at the surface of the electrode ($n = 1$), $F$ is the Faraday constant, $A$ is the area of the electrode, $k^*$ is the standard rate constant for the process of heterogeneous electron transfer, $c_o$ and $c_r$ are the concentrations of the oxidized and reduced forms of the redox-active species at the surface of the electrode (these concentrations are equal to the bulk concentrations in the absence of mass transport limitations), $\alpha$ is the transfer coefficient (defined below), $f = FRT$, $T$ is temperature, $E$ is the potential of the electrode, and $E^{\circ}$ is the formal reduction potential of the redox-active species. The transfer coefficient can be understood as follows: for an electrochemical process at an electrode with a free energy of activation of $\Delta G$, at $E = 0$ V, a change in the potential of the electrode causes a change in the activation free energy of $\Delta GFE$. For solutions containing only oxidized ($c_r = 0$) or reduced ($c_r = 0$) forms of the redox species, eq 2 can be simplified to eqs 3 and 4. We have used eq 3 to

$$i_o = nFk^*c_r e^{-\alpha FE - E^{\circ}} c_r = 0 \quad (3)$$

$$i_r = -nFk^*c_o e^{(1-\alpha)FE - E^{\circ}} c_o = 0 \quad (4)$$

analyze the cathodic current in Figure 8b. A transfer coefficient (see eq 3) of 0.18 and an apparent heterogeneous rate constant of $5.6 \times 10^{-7} \text{ cm/s}$ were obtained from a Tafel plot (not shown) of the data. These values are in approximate agreement with measurements reported by Miller and co-workers for SAMs of $\text{CH}_2(\text{CH}_2)_{10}\text{S}$ and $\text{Fe(CN)}_6^{3-}$ in 0.1 M KCl; these authors report a transfer coefficient of 0.2 and an apparent heterogeneous rate constant of $1.3 \times 10^{-7} \text{ cm/s}$.

Grooves micromachined in the SAM caused an increase in Faradaic current relative to that passed at gold covered with a SAM of $\text{CH}_2(\text{CH}_2)_{10}\text{S}$, as seen by the cyclic voltammograms of Figure 8c. Both cyclic voltammograms have sigmoidal shapes, in contrast to Figure 8a which has the well-defined anodic and cathodic peaks characteristic of (semi-infinite) diffusion-controlled electron transfer at a macroscopic electrode. The limiting cathodic current ($i_{lim}$) for the array of 8-μm-wide grooves was measured as a function of the scan rate between 10 and 100 mV/s; the obtained exponent of 0.12 ($i_{lim} \propto v^{0.12}$) indicates that mass transport to the microgroove electrodes is more rapid than mass transport to the macroscopic electrodes and can no longer be described by planar diffusion (see above).

The cyclic voltammogram measured using the array of 1-μm-wide grooves was obtained after the surfaces of the grooves were electrochemically conditioned by cycling the potential between $-0.4$ and $+1.4$ V in an aqueous solution of 0.1 M $\text{H}_2\text{SO}_4$. If this procedure was not performed, the plateau in current at negative potentials was generally less pronounced than that shown in Figure 8c: the cathodic current increased monotonically with more negative potentials. This electrochemical behavior was reversed in aqueous solutions of $\text{Fe(CN)}_6^{3-}$: no current was measured at negative potentials, while a monotonic increase in anodic current was measured at positive potentials. The same samples did not show Faradaic currents in aqueous solutions of electrolyte without redox-active molecules. The cyclic voltammograms measured using arrays of 8-μm-wide grooves did not require electrochemical conditioning in aqueous solutions of 0.1 M $\text{H}_2\text{SO}_4$.

We interpret the absence of peaks and the presence of plateaus in the cyclic voltammograms measured using arrays of microgrooves to indicate a near-steady-state concentration of redox-active solutes was maintained in the film of liquid near the electrode boundary layer during the forward and reverse voltage scans. The limiting currents observed in Figure 8c were determined, we believe, by the maximum rate of mass transport of ions from bulk solution to the surface of the electrode.

Mass transport-limited currents, $i(t)$, at band-type microelectrodes can be estimated by using eqs 5 and 6, where $\tau$ is dimensionless time ($\tau = D/w^2$), $t$ is time, $w$ is the width of the band electrode, $D$ is the diffusivity of the redox-active species, $c_o$ is the bulk concentration of redox-active species, $L$ is the length of the band electrode, and $\gamma = 0.577$. Equations 5 and 6 are approximate and have been found to agree with numerical simulations to within 1.3%.

For an array of 20 grooves, each 1 μm wide ($w = 1 \times 10^{-6} \text{ cm}$), 1 cm long ($L = 20 \times 1 \text{ cm} = 20 \text{ cm}$), and contacting a 1 mM solution of $\text{Fe(CN)}_6^{3-}$ ($D = 6.1 \times 10^{-6} \text{ cm}^2/\text{s}$), $c_o = 1 \times 10^{-4} \text{ mol/cm}^3$, we calculate $\gamma = 2440$ and, using eq 5, $i(t) = 4 s \approx 6.5 \mu\text{A}$. In comparison, the experimentally measured current was $\sim 3 \mu\text{A}$ (Figure 8c).

$$i(t) = \frac{nFDEc_o}{4(\pi t)^{1/2}} \ln(64\gamma t) \quad \tau > 2/5 \quad (5)$$

$$i(t) = \frac{1}{(\pi t)^{1/2}} + 1 \quad \tau < 2/5 \quad (6)$$

(37) Miller et al. used SAMs formed from aqueous solutions of 16-mercaptohexadecanol (in the presence of a cationic surfactant); the rate constants were measured at scan rates of 50 V/s in aqueous solutions of 0.1 M KCl. Factors such as the type and concentration of electrolyte, the nature of the head group of the SAM, and the rate at which the potential is scanned have been reported to influence the apparent rate constants. All these factors can plausibly contribute to the difference in the value of the rate constants measured by us and by Miller et al.


(40) Four seconds corresponds to the time taken to scan the potential 200 mV (from 0.2 to 0.4 V in Figure 8c) at 50 mV/s.
Combining Micromachining and Molecular Self-Assembly

**Figure 9.** Cyclic voltammograms measured in aqueous solutions of 2 mM Ru(NH₃)₃Cl₃, 10 mM K₂HPO₄ (pH 7.0), and 0.1 M NaClO₄: (a) macroscopic film (~0.2 cm²) of gold evaporated onto a glass microscope slide (50 mV/s); (b) film of gold (~1 cm²) covered with a SAM of CH₃(CH₂)₁₅S (10 mV/s); (c) array of 8-μm-wide grooves in an aqueous solution of 2 mM Ru(NH₃)₃Cl₃ was in good agreement with the calculated (using D: 7.1 x 10⁻⁶ cm²/s, τ: 190, and, using eq 5: 2.0 x 10⁻⁶ cm²/s). The smaller value of the measured current may arise because eq 5 describes mass transport to the surface of the vertical walls of the wires. We calculated the working area of the electrode by approximating the shape of the wire as a single, horizontal 0.2-μm-wide band electrode and used eq 5 to estimate the magnitude of the mass transport-limited current to its surface. Electrodes were measured by placing a 10–20-μL drop of aqueous solution (containing the redox-active species and electrolyte) on the gap and inserting a silver wire (reference electrode) into the drop.

**Figure 10.** Cyclic voltammograms of a wire electrode: 100 nm x 13 mm x 1 μm; shown in Figure 5: (a) 1 mM ferrocene. 0.1 M TBAPF in acetonitrile; (b) aqueous solution of 1 mM K₃Fe(CN)₆, 10 mM K₂HPO₄ (pH 7.0), and 0.1 M NaClO₄. The potential was scanned at 50 mV/s. Note the different scales of current on the vertical axes.
Because the mass flux to a disk or hemispherical electrode increases inversely with the radius of the electrode, and because the rate of heterogeneous electron transfer is independent of the radius of the electrode, kinetic effects become the rate-determining step for small electrodes. The size of an electrode, \( r \), corresponding to the crossover from mass transport-limited currents to kinetically limited currents can be estimated from \( r = D/k^2 \), where \( D \) is the diffusivity and \( k^2 \) is the standard rate constant for the heterogeneous electron transfer process. For Fe(CN)⁶³⁻⁴ and gold electrodes (\( D = 6.1 \times 10^{-6} \text{ cm}^2/\text{s} \) and \( k^2 = 1.0 \times 10^{-10} \text{ to } 6.0 \times 10^{-12} \text{ cm}^2/\text{s} \)), we estimated \( r \approx Dk^2 \) to range from 1 to 3 \( \mu \text{m} \), a value that is comparable in magnitude to the size of the gap electrodes estimated from scanning electron microscopy (Figure 6). This calculation supports the hypothesis that the kinetics of electron transfer are important in determining the shape of the cyclic voltammograms obtained for gap electrodes, as seen in Figure 11. The electrochemically active area of the gap electrode was estimated from the experimentally observed currents by assuming the currents in Figure 11b,c to be governed by the rate of heterogeneous electron transfer (and not mass transport). Recent work by Smith and White indicates that at spherical electrodes with diameters \( \approx 0.1 \mu\text{m} \), the violation of electroneutrality—even in the presence of excess supporting electrolyte—can lead to the appearance of a kinetically limited wave shape even when the electrochemical reaction continues to be transport-limited. We do, however, treat our data as indicative of kinetically limited currents and compare our results with previous reports.

We have used eqs 3 and 4 to interpret the cyclic voltammograms shown in Figure 11. Figure 12 is a plot of \( \ln(i) \) versus \( E - E'' \), using cyclic voltammograms in Figure 11 for gap electrodes measured in aqueous solutions of 1 mM \( \text{K}_3\text{Fe(CN)}_6 \) or 1 mM \( \text{K}_4\text{Fe(CN)}_6 \), 10 mM \( \text{K}_2\text{HPO}_4 \), and 0.1 M NaClO₄.

Because the flux to a disk or hemispherical electrode increases inversely with the radius of the electrode, and because the rate of heterogeneous electron transfer is independent of the radius of the electrode, kinetic effects become the rate-determining step for small electrodes. The size of an electrode, \( r \), corresponding to the crossover from mass transport-limited currents to kinetically limited currents can be estimated from \( r = D/k^2 \), where \( D \) is the diffusivity and \( k^2 \) is the standard rate constant for the heterogeneous electron transfer process. For Fe(CN)⁶³⁻⁴ and gold electrodes (\( D = 6.1 \times 10^{-6} \text{ cm}^2/\text{s} \) and \( k^2 = 1.0 \times 10^{-10} \text{ to } 6.0 \times 10^{-12} \text{ cm}^2/\text{s} \)), we estimated \( r \approx Dk^2 \) to range from 1 to 3 \( \mu\text{m} \), a value that is comparable in magnitude to the size of the gap electrodes estimated from scanning electron microscopy (Figure 6). This calculation supports the hypothesis that the kinetics of electron transfer are important in determining the shape of the cyclic voltammograms obtained for gap electrodes, as seen in Figure 11. The electrochemically active area of the gap electrode was estimated from the experimentally observed currents by assuming the currents in Figure 11b,c to be governed by the rate of heterogeneous electron transfer (and not mass transport). Recent work by Smith and White indicates that at spherical electrodes with diameters \( \approx 0.1 \mu\text{m} \), the violation of electroneutrality—even in the presence of excess supporting electrolyte—can lead to the appearance of a kinetically limited wave shape even when the electrochemical reaction continues to be transport-limited. We do, however, treat our data as indicative of kinetically limited currents and compare our results with previous reports.

We have used eqs 3 and 4 to interpret the cyclic voltammograms shown in Figure 11. Figure 12 is a plot of \( \ln(i) \) versus \( E - E'' \), using cyclic voltammograms in Figure 11 for gap electrodes measured in aqueous solutions of 1 mM \( \text{K}_3\text{Fe(CN)}_6 \) or 1 mM \( \text{K}_4\text{Fe(CN)}_6 \), 10 mM \( \text{K}_2\text{HPO}_4 \), and 0.1 M NaClO₄.
Because groove and gap electrodes require the SAM to be electrically insulating, and because SAMs of CH$_2$-\(\text{CH}_2\)$_{18}$SO do not insulate an underlying film of gold from a contacting solution of ferrocene in acetonitrile, these electrodes do not function with ferrocene in acetonitrile; the electrodes do, however, function in aqueous systems. The "microwire" electrodes, in contrast, can be used in both aqueous and nonaqueous solvents. The gap electrodes offer the potential to detect the presence of redox-active analytes in very small aqueous volumes of liquid (\(\sim 1\) \(\mu\)L).

The above voltammetric characteristics of these microelectrodes and the simplicity of the wet chemical method used to achieve their microfabrication offer an attractive approach for design, creation, and prototyping of micrometer-scale, electrically-conducting structures for electroanalytical and bioelectrochemical studies.

### Experimental Section

#### Materials

Titanium (99.999+%), Au (99.999+%), ferrocene, CH$_3$CH$_2$SH, and K$_2$Fe(CN)$_6$ were obtained from Aldrich. HO$_2$-CH$_2$SH and K$_2$Fe(CN)$_6$ were obtained from Mallinckrodt. Ru(NH$_3$)$_6$Cl$_3$ was obtained from Johnson Matthey. KOH and KCN were obtained from Fisher Scientific. All solvents and reagents were used as received. The water was distilled and deionized before use.

#### Preparation of Gold Films

Glass microscope slides (VWR) were cleaned in piranha solution (30% H$_2$O$_2$ and 70% H$_2$SO$_4$) until strongly orange in color. The solution was changed from that measured using both electrodes in the gap to a contacting solution of ferrocene in acetonitrile. Both 10 mM K$_2$HPO$_4$ and 0.1 M NaClO$_4$ were added to the Piranha solutions to make a solution of ferrocene in acetonitrile, these gold electrodes were washed in ethanol and dried in an oven prior to placement in the evaporator chamber. These gold wafers were cleaned using a hand-operated semi-automatic stage.

#### Fabrication of Microstructures

A "Microgroove" Electrode. SAMs were formed on the surfaces of the evaporated gold films covered with SAMs of CH$_3$CH$_2$SH and K$_2$Fe(CN)$_6$ were obtained from Mallinckrodt. Ru(NH$_3$)$_6$Cl$_3$ was obtained from Johnson Matthey. KOH and KCN were obtained from Fisher Scientific. All solvents and reagents were used as received. The water was distilled and deionized before use.

#### Electroanalytical and Bioelectrochemical Studies

In this paper we report wet chemical methods to fabricate microstructures from gold. Cyclic voltammetry was used to demonstrate that these microstructures can function as microelectrodes. Three types of microstructures were prepared: grooves, wires, and gaps. Each of these microstructures had at least one dimension that was in the \(\sim 1\mu m\) range or less. Because of their small dimensions, these electrodes showed electrochemical responses during cyclic voltammetry that reflected non-planar diffusion of redox-active species in solution to the surface of the electrode.

The grooves and supported wire electrodes produced cyclic voltammograms with little hysteresis and well-defined limiting currents; the measured limiting currents were consistent with estimates of the rate of mass transport of redox-active species to the electrode. Currents at "microgap" electrodes do not, however, appear to be limited by mass transport. Instead, the kinetics of electron transfer from the redox species to the gold appear to determine the voltammetric behavior of the electrodes.

### Table 3. Apparent Transfer Coefficients and Radii of Gap Electrodes Measured in Aqueous Solutions of Either 1 mM K$_2$Fe(CN)$_6$ or 1 mM K$_2$Fe(CN)$_6$ and Both 10 mM K$_2$HPO$_4$ and 0.1 M NaClO$_4$

<table>
<thead>
<tr>
<th>Fe(CN)$_6^{3-}$</th>
<th>Fe(CN)$_6^{4-}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>slope</td>
<td>-0.5</td>
</tr>
<tr>
<td>intercept</td>
<td>0.9</td>
</tr>
<tr>
<td>transfer coeff.</td>
<td>0.1</td>
</tr>
<tr>
<td>apparent radius</td>
<td>2.3-5.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fe(CN)$_6^{3-}$</th>
<th>Fe(CN)$_6^{4-}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>slope</td>
<td>-5.5</td>
</tr>
<tr>
<td>intercept</td>
<td>6.9</td>
</tr>
<tr>
<td>transfer coeff.</td>
<td>-20.0</td>
</tr>
<tr>
<td>apparent radius</td>
<td>2.0-4.8</td>
</tr>
</tbody>
</table>

Note that the number of redox-active species that undergo electron transfer at the electrode during the course of the experiment is negligible compared to the number of those present within the 10-20\(\mu L\) drop of solution. The measured cyclic voltammograms do not reflect, therefore, an exhaustion of the redox-active species within the drop.
waxy residue on the surface of the gold and incomplete etching of the areas of the gold film covered with a monolayer of HO-(CH₂)₄S. (v) Regions of the gold film covered with a monolayer formed from HO(CH₂)₂SH were selectively etched by immersing the entire gold film in an aqueous solution of 1 M KOH and 0.1 M KCN that was sparged with O₂ and stirred vigorously. The time required to etch the sample to the glass substrate was typically ~45 min. (vi) The wire was inspected for defects using optical microscopy and checked to ensure that it was electrically conducting.

C. Microgap Electrode. The starting point for the preparation of microgap electrodes was a microwire (described above) connected between two macroscopic electrical contact pads. (i) The surface of the microwire was electrochemically cleaned by immersing the wire in an aqueous solution of 0.1 M H₂SO₄ and cycling the electrical potential between -0.4 and +1.4 V. (ii) The electrochemically cleaned microwire was immersed in an ethanolic solution containing ~10 mM CH₃(CH₂)₄SH for at least 24 h. (iii) Cyclic voltammetry in 1 mM K₃Fe(CN)₆ (pH 7, 5 mM KH₂PO₄, 5 mM K₂HPO₄, 0.1 M NaClO₄) was used to confirm that an electrically insulating SAM of CH₃(CH₂)₄S had formed on the surface of the microwire. (iv) A surgical scalpel was used to micromachine a 1-μm-wide gap into the microwire. The resistance between the two electrical contact pads was measured to increase from approximately 2450 Ω before the gap was machined to in excess of 20 MΩ after machining of the gap. (v) The electrochemical behavior of the electrodes was measured by placing a 10–20-μL drop of the solution containing the redox-active species on the surface of the glass slide on which the electrodes were supported. A silver wire was inserted into the drop and was used as a reference electrode.

Cyclic Voltammetry. All solutions were deaerated by bubbling Ar prior to use. The reference electrode was either a saturated calomel electrode or a silver wire (diameter 0.1 mm). Before performing electrochemical measurements, the surface oxides were removed from the silver wire using an abrasive paper. Solutions were not stirred during measurement of the cyclic voltammograms. Measurements were performed using a signal generator (Princeton Applied Research Model 175 universal programmer) used in conjunction with a three-electrode potentiostat (Princeton Applied Research Model 174A polarographic analyzer).

Acknowledgment. Partial support of this research was provided by the Advanced Projects Research Agency and the Office of Naval Research. The authors thank Steven Bergens, Hans Biebuyck, and Watson Lees for useful suggestions and interesting discussions.