Electrochemical Cleaning Protocol

Last updated: 03/09/2022. Written by Yao Wu, Ph.D.

This cleaning protocol combines mechanical polishing of gold electrodes with chemical and electrochemical cleaning, to produce clean electrodes with smooth surfaces for effective sensor fabrication.

Step 1. Polish each electrode on a cloth pad using a 0.05 µm alumina slurry for 2 min. If your electrodes need coarser polishing, you can use 1 µm (particle diameter) diamond suspension paste first, then the 0.1 µm diamond suspension, and last polish with 0.05 µm alumina slurry, rinsing and sonicating in DI water between polishing steps. Both pastes should always be available in our lab. If you can’t find them, reach out to Netz or the person in charge of making supply purchases.

Step 2. Rinse polished electrodes with DI water and sonicate them in DI water for 2 min.

Step 3. Immerse freshly polished electrodes into a beaker containing 20% gold cleaning solution in water for 60 s.

Step 4. Quickly transfer the electrodes to another beaker with DI water to stop the reaction.

Step 5. Sonicate the electrodes in 1:1 ratio ethanol and 0.5 M NaOH (aqueous) mixture for 10 min.

Step 6. During the last step, turn ON the CHI 1040C potentiostat and open the software. Go to the menu item Setup, and run a hardware test twice.

Step 7. Once the hardware test shows OK labels for all metrics, run an initial CV scan (the parameters do not matter) without connecting any electrodes. This is to ensure that the open circuit voltage of the instrument is reset before you perform any measurements.

Step 8. Repeat step 7 after connecting one working, and reference/counter electrodes and placing them in phosphate-buffered saline.

Step 9. If you see any connection problems or potentiostat issues in your voltammograms, please don’t be lazy, disconnect all the electrodes and restart the CHI program. Then run steps 6-8 once again. It is highly recommended that you apply step 6-8 for any experiments that you run with CHI potentiostats. Occasionally the potential applied to the WEs is not correct during the first run (faulty potentiostat behavior). If this potential is too high on the WEs, it could generate a lot of gold oxide on the electrode surfaces, which will take you additional polishing to remove and make sensor surfaces smooth again.

Step 10. If everything looks good, connect all the working electrodes in the multichannel setting.

Step 11. Run an initial desorption scan in 0.5 M NaOH. The potential window should be (-0.8 to -1.4 V vs Ag|AgCl). See an example run in the next page.
Step 12. Rinse the electrodes with DI water. Then run gold oxidation and reduction CV scans in 0.5 M H$_2$SO$_4$. Parameters are shown the window below. If your electrodes are smooth, the reduction peak current in 0.5 M H$_2$SO$_4$ at 4 V/s is around 5x10$^4$ A using 1.6 mm commercial gold disc electrodes.
Step 13. Repeat step 12 in a fresh solution of 0.5 M H$_2$SO$_4$.

Step 14. If you are using the commercial disc electrodes, you could end your cleaning here and proceed with aptamer deposition. The last step should be a CV run in 0.05 M H$_2$SO$_4$ at 0.1 V/s to estimate the area under the gold oxide reduction peak. The example below shows this step on 16 electrodes, with excellent reproducibility across them.

![Image of CV graph]

Example run for Step 14

Step 15. If you work with microfabricated electrodes and want to increase their surface area, you can run chronoamperometric pulsing to roughen the electrodes. Set up 20 repetitive runs by clicking control repetitive runs icon in the menu, this step would help with increasing surface area uniformly. The correct parameters are shown in the window below.

![Image of chronoamperometric pulsing window]

Example run for Step 15
Standard Operating Procedure

Step 16. After 20 repetitive chronoamperometry runs, perform a CV scan in 0.05 M H₂SO₄ at 0.1 V/s. The area after 20 cycles of roughing increases by ~20%. Example CVs are included below:

![Example CV for Step 16]

**Example run for Step 16**

**Additional Notes:**

If you see other peaks (not the characteristic peaks of gold oxidation (~1.1 V) or reduction peaks (~0.9 V)) with the area scan in 0.05 M H₂SO₄, repeat the cleaning procedure in 0.5 M H₂SO₄ 2 times and scan electrodes again in 0.05 M H₂SO₄. If the other peaks are still there, you could consider sonicating the electrodes in 1:1 ratio ethanol and 0.5 M NaOH mixture for another 5 min. Sometimes 20% gold cleaning solution could overly oxidize your surface, especially if you immerse your electrodes in the solution for more than 60 s.

Unless the surface is already smooth, mechanical polishing cannot be avoided. It is not recommended cleaning your electrodes without mechanical polishing on diamond suspension or alumina slurry. However, for smooth electrodes, it might be okay to skip this step. Rough surfaces can be re-smoothed by mechanical polishing + chemical + e-chemical treatment. If surfaces are rough (i.e., roughness factor > 2), polishing on 1 µm diamond suspension + 2 min sonication + 0.1 µm diamond suspension + 2 min sonication + chemical treatment + e-cleaning a few times can bring down roughness factor. If these don’t work, then polishing on a 1200/P2500 silicon carbide grinding paper first, followed by above steps.

**Commercial gold cleaning solution (0.1 – 1 % thiourea and 5 – 10 % sulfuric acid, SIGMA #667978).** This solution oxidizes the gold surface according to equation 1 [1]. Incubation in ethanol/NaOH mixture removes any gold oxides remaining from the fabrication process according to equation 2 [2].

\[
Au + 2 \text{CS(NH}_2\text{)}_2 \rightleftharpoons Au(\text{NH}_2\text{CSNH}_2)^{2+} + e^- \quad \text{eq. 1}
\]

\[
Au_2O_3 + 3 \text{CH}_3\text{CH}_2\text{OH} \rightleftharpoons 2Au + 3 \text{CH}_3\text{CHO} + 3\text{H}_2\text{O} \quad \text{eq. 2}
\]
Tips: Electrode roughness, probe coverage, and electron transfer rate can be calculated.

1. **Gold Substrate Characterization: electrode roughness**

The real surface area of each electrode was estimated on the basis of the amount of charge consumed during the reduction of the gold surface oxide in 0.05 M H$_2$SO$_4$ using a reported value of 400 μC cm$^{-2}$.

With this cleaning protocol, the roughness of electrodes well controlled around roughness factor ~1.2 if we assume the roughness of smooth surface is 1. With 20 scans chronoamperometry roughing applied, the roughness factor becomes ~1.6. If you want to increase your area even more, you could potentially apply more cycles of chronoamperometry roughing step. However, you are going to test out whether the sensor performance is better with additional chronoamperometry roughing.

![Electrode Roughness and Probe Coverage Example](image)

<table>
<thead>
<tr>
<th>Electrode</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>R5</th>
<th>R6</th>
<th>R7</th>
<th>R8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au/μC (Reduction peak charge)</td>
<td>11.04</td>
<td>12.37</td>
<td>12.15</td>
<td>12.44</td>
<td>12.65</td>
<td>12.77</td>
<td>12.5</td>
<td>13</td>
</tr>
<tr>
<td>Area Ah(400) (Peak Charge/400 μC)</td>
<td>0.02395</td>
<td>0.025925</td>
<td>0.030625</td>
<td>0.0311</td>
<td>0.034125</td>
<td>0.03193</td>
<td>0.03125</td>
<td></td>
</tr>
<tr>
<td><strong>Roughness of electrode (1.6 mm in diameter)</strong></td>
<td>1.485370223</td>
<td>1.58826402</td>
<td>1.523913</td>
<td>1.545731606</td>
<td>1.698099</td>
<td>1.5882</td>
<td>1.550636</td>
<td>1.617237</td>
</tr>
<tr>
<td><strong>Probe concentration</strong></td>
<td>2 μM</td>
<td>2 μM</td>
<td>2 μM</td>
<td>2 μM</td>
<td>2 μM</td>
<td>2 μM</td>
<td>2 μM</td>
<td>2 μM</td>
</tr>
<tr>
<td>Reduction Peak Charge at CV Scan Rate 0.02v/s</td>
<td>55.1500</td>
<td>58.4150</td>
<td>48.2150</td>
<td>52.9500</td>
<td>52.6150</td>
<td>54.5150</td>
<td>58.0450</td>
<td></td>
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<tr>
<td>Reduction Peak Charge at CV Scan Rate 0.05v/s</td>
<td>51.9100</td>
<td>53.6400</td>
<td>44.0150</td>
<td>56.7700</td>
<td>46.3050</td>
<td>54.9000</td>
<td>51.6350</td>
<td>54.2500</td>
</tr>
<tr>
<td>Reduction Peak Charge at CV Scan Rate 0.1v/s</td>
<td>48.8600</td>
<td>53.1700</td>
<td>45.8500</td>
<td>50.7300</td>
<td>45.7200</td>
<td>57.8400</td>
<td>47.5300</td>
<td>55.0700</td>
</tr>
<tr>
<td>Average charge (μC)</td>
<td>51.92333</td>
<td>55.07333</td>
<td>46.23667</td>
<td>53.476666</td>
<td>40.04</td>
<td>58.1907</td>
<td>51.21667</td>
<td>55.70333</td>
</tr>
</tbody>
</table>

2. **Probe Coverage Determination**

The density of electroactive DNA probes on the electrode surface, $\Gamma$, was determined by integrating the charge under the MB reduction peak in CV scans collected at slow scan rates.

$$\Gamma = \frac{Q}{nFA}$$

where $Q$ is the integrated charge of the reduction peak in the CV scans, $n$ is the number of electrons transferred per redox event ($n = 2$ for MB), $F$ is the Faraday's constant, and $A$ is the real electrode area. $\Gamma$ for each of the six systems is presented as an average value from CVs recorded at three different scan rates (20, 50 and 100 mV s$^{-1}$).

3. **Electron Transfer Rate Determination**

Values of CV peak separation ($\Delta E_p = E_{pa} - E_{pc}$) as a function of increasing fast scan rate ($v$) reflect the electron transfer rate $K_s$. According to Laviron equation, when $\Delta E_p > 200/n$ mV, a graph of $\Delta E_p$ versus log $v$ yields a straight line. \[3\]

$$\log K_s=\alpha \log(1-\alpha) + (1-\alpha) \log \alpha-\log \frac{R F}{n F v} - \log \frac{(1-\alpha)nF\Delta E_p}{2.3RT}$$ \[1.4\]

Where $K_s$ is the electron-transfer rate constant (s$^{-1}$), $\alpha$ is the electrontransfer coefficient, $v$ is the CV potential scan rate (V/s), and $\Delta E_p$ is the difference between the anodic potential and cathodic potential (V). $\alpha$ can be determined from the slope of the straight line, and $K_s$ can be calculated with the help of the intercept.
Please refer to the attached excel template for your calculations.

References:

