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.

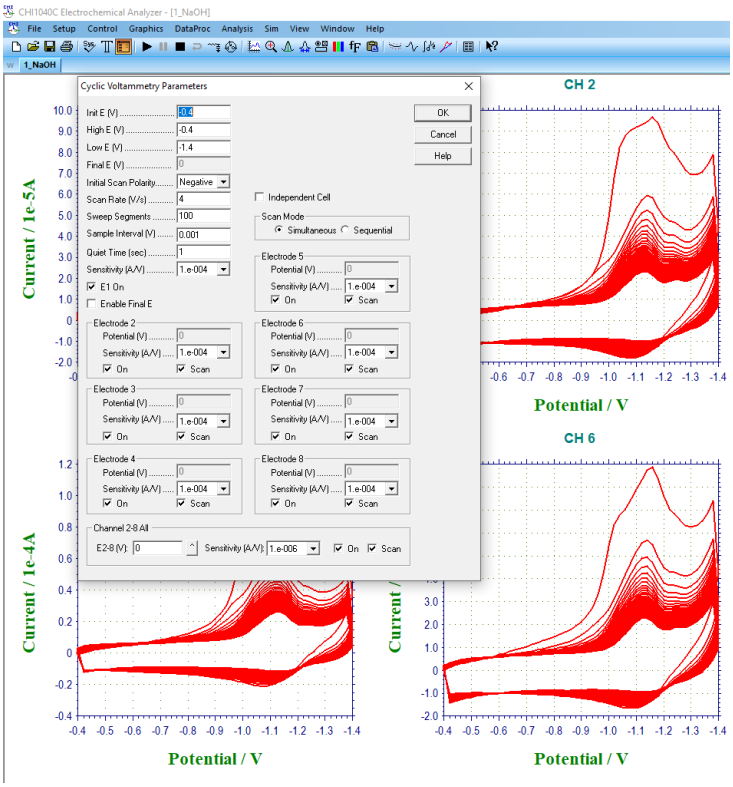
Step 7. Once the hardware test shows OK labels for all metrics, run an initial CV scan (the parameters do not mater) without connecting any electrodes. This is to ensure that the open circuit voltage of the instrument is reset before you perform any measurements.

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.

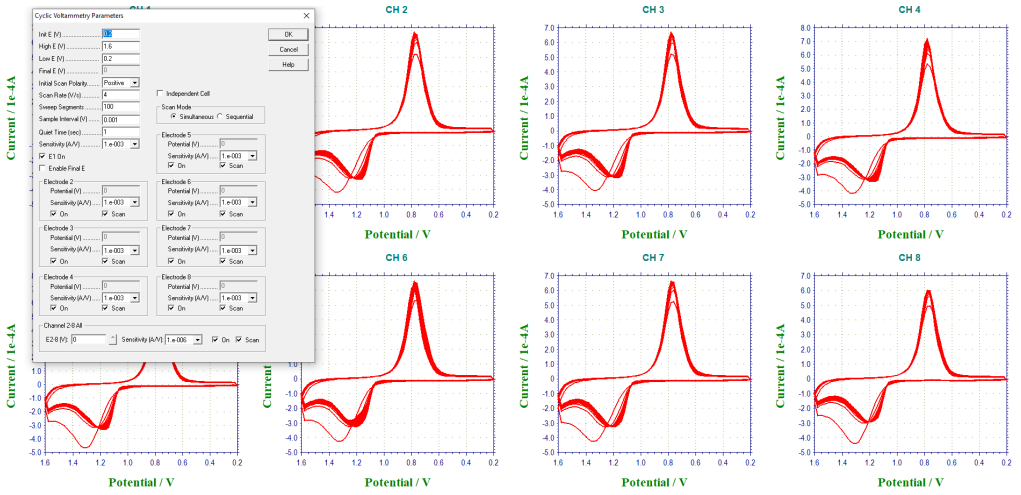
Step 10. If everything looks good, connect all the working electrodes in the multichannel setting.

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.



Example run for Step 11

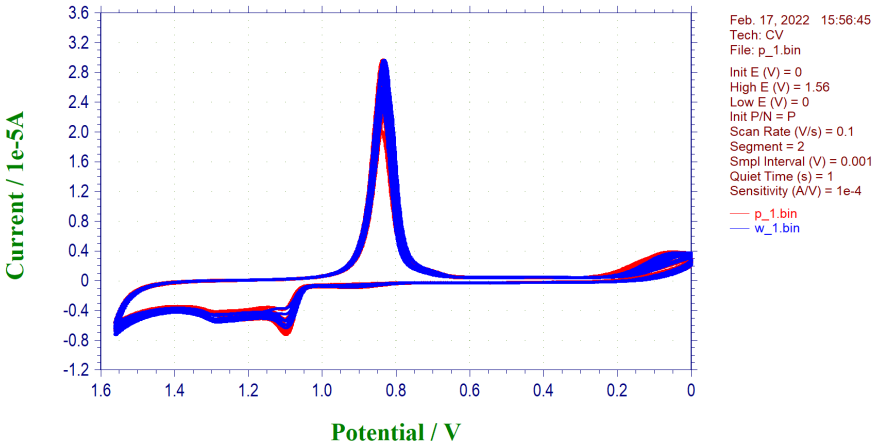
Step 12. Rinse the electrodes with DI water. Then run gold oxidation and reduction CV scans in 0.5 M H_2SO_4. Parameters are shown the window below. If your electrodes are smooth, the reduction peak current in 0.5 M H_2SO_4 at 4 V/s is around $5x10^4$ A using 1.6 mm commercial gold disc electrodes.



Example run for Step 12

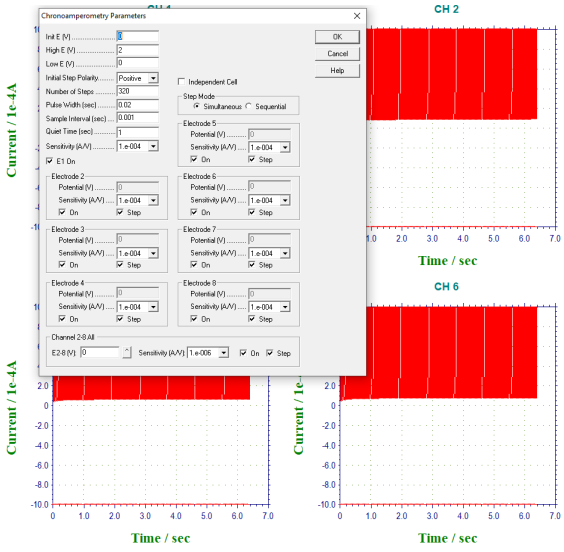
Step 13. Repeat step 12 in a fresh solution of 0.5 M H_2SO_4.

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_2SO_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.



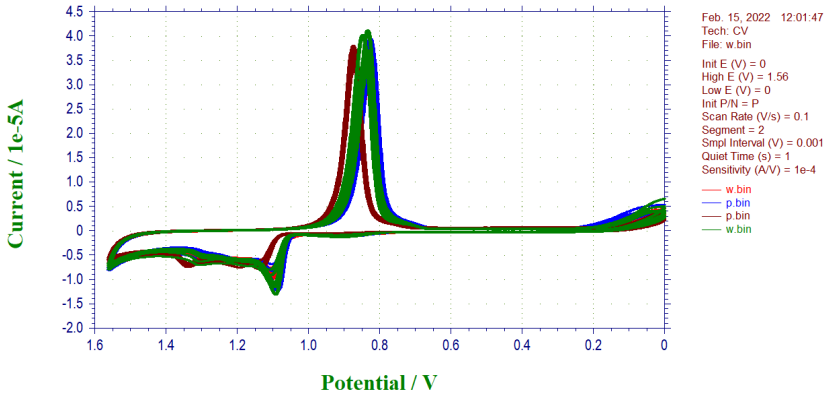
Example run for Step 14

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.



Example run for Step 15

Step 16. After 20 repetitive chronoamperometry runs, perform a CV scan in 0.05 M H_2SO_4 at 0.1 V/s. The area after 20 cycles of roughing increases by ~20%. Example CVs are included below:



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 H_2SO_4, repeat the cleaning procedure in 0.5 M H_2SO_4 2 times and scan electrodes again in 0.05 M H_2SO_4. 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].**

$$Au + 2\,CS(NH_2)_2 \rightleftharpoons Au(NH_2CSNH_2)^{2+} + e^- \qquad \text{eq. 1}$$

$$Au_2O_3 + 3\,CH_3CH_2OH \rightleftharpoons 2\,Au + 3\,CH_3CHO + 3\,H_2O \qquad \text{eq. 2}$$

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_2SO_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 Examplar								
Electrode #	#1	#2	#3	#4	#5	#6	#7	#8
Au/μC (Reduction peak charge)		11.94	12.77	12.25	12.44	13.65	12.77	12.5	13
Area Ah(400) (Peak Charge/400 μC)		0.02985	0.031925	0.030625	0.0311	0.034125	0.03193	0.03125	0.0325
Roughness of electrode (1.6 mm in diameter)		1.485370223	1.588624602	1.523935	1.547571656	1.698099	1.58862	1.555036	1.617237
Probe concentration	2uM		2uM	2uM	2uM	2uM	2uM	2uM	2uM
Reduction Peak Charge at CV Scan Rate_0.02v/s		55.0000	58.4100	48.2100	52.9300	52.1000	61.7600	54.5100	58.0600
Reduction Peak Charge at CV Scan Rate_0.05v/s		51.9100	53.6400	44.6500	56.7700	49.3000	54.9900	51.6100	54.2500
Reduction Peak Charge at CV Scan Rate_0.1v/s		48.8600	53.1700	45.8500	50.7300	45.7200	57.8400	47.5300	55.0700
Average charge (μC)		51.9233	55.07333333	46.23667	53.47666667	49.04	58.1967	51.21667	55.79333
Surface Probe Coverage Γaptamer(10e12molecules,		5.437363536	5.392381348	4.719332	5.37494569	4.492085	5.6982	5.123083	5.366228
			Avg.	Stdv.					
			5.301647061	0.306709					

2. Probe Coverage Determination

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

$$\Gamma = Q/nFA$$

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. *Γ* 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}).

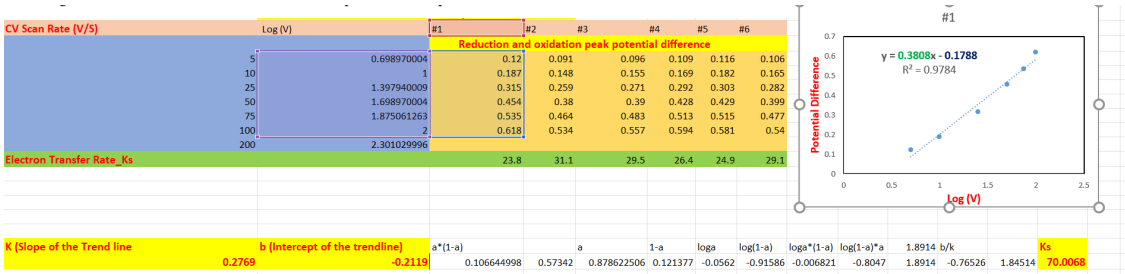
3. Electron Transfer Rate Determination

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 *Ks*. According to Laviron equation, when $\Delta E_p > 200/n$ mV, a graph of ΔE_p versus log v yields a straight line. **[3]**

$$\text{Log } Ks = \alpha \log(1-\alpha) + (1-\alpha) \log \alpha - \log\left(\frac{RT}{nFv}\right) - \frac{\alpha(1-\alpha)nF\Delta Ep}{2.3RT} \qquad (1.4)$$

Where *Ks* is the electron-transfer rate constant (s^{-1}), α is the electrontransfer coefficient, v is the CV potential scan rate (V/s), and ΔE_p is the difference between the anodic potential and cathodic potential (V). α can be determined from the slope of the straight line, and *Ks* can be calculated with the help of the intercept.



CV Scan Rate (V/S)	Log (V)	#1	#2	#3	#4	#5	#6
		Reduction and oxidation peak potential difference					
5	0.698970004	0.12	0.091	0.096	0.109	0.116	0.106
10	1	0.187	0.148	0.155	0.169	0.182	0.165
25	1.397940009	0.315	0.259	0.271	0.292	0.303	0.282
50	1.698970004	0.454	0.38	0.39	0.428	0.429	0.399
75	1.875061263	0.535	0.464	0.483	0.513	0.515	0.477
100	2	0.618	0.534	0.557	0.594	0.581	0.54
200	2.301029996						
Electron Transfer Rate_Ks		23.8	31.1	29.5	26.4	24.9	29.1

K (Slope of the Trend line	b (Intercept of the trendline)	a*(1-a)	a	1-a	loga	log(1-a)	loga*(1-a)	log(1-a)*a	1.8914 b/k		Ks
0.2769	-0.2119	0.106644998	0.57342	0.878622506	0.121377	-0.0562	-0.91586	-0.006821	-0.8047	1.8914 -0.76526	1.84514 70.0068

Please refer to the attached excel template for your calculations.

References:

(1) Carvalhal, R. F.; Sanches Freire, R.; Kubota, L. T., Polycrystalline gold electrodes: A comparative study of pretreatment procedures used for cleaning and thiol self-assembly monolayer formation. *Electroanalysis* **2005**, *17* (14), 1251-1259.

(2) Groenewald, T., The dissolution of gold in acidic solutions of thiourea. *Hydrometallurgy* **1976**, *1* (3), 277-290.

(3) Laviron, E. General expression of the linear potential sweep voltammogram in the case of diffusionless electrochemical systems. *J. Electroanal. Chem. Interfacial Electrochem.* **1979**, 101,19−28.