Published in final edited form as:

Anal Chem. 2017 March 07; 89(5): 2739–2746. doi:10.1021/acs.analchem.6b02916.

Electrostatic Ion Enrichment in an Ultrathin-Layer Cell with a Critical Dimension between 5 and 20 nm

Jin Lu and Bo Zhang*

Department of Chemistry, University of Washington, Seattle, Washington 98195-1700, United States

Abstract

Electrostatic interactions play an essential role in many analytical applications including molecular sensing and transport studies using nanopores and separation of charged species. Here, we report the voltammetric quantification of electrostatic ion enrichment in a 5–20 nm thin electrochemical cell. A simple lithographic micro/ nanofabrication process was used to create ultrathin-layer cells (UTLCs) with a critical dimension (i.e., cell thickness) as small as 5 nm. The voltammetric response of a UTLC was found to be largely dominated by the electrostatic interaction between charges on the cell walls and the redox species. We show that the ultrasmall cell dimension yielded a 100–300-fold enrichment for cationic redox species. An interesting surface adsorption effect was also demonstrated.

Graphical abstract

Electrostatic interactions play an essential role in numerous analytical applications including molecular sensing and transport using nanopores^{1–6} and separation of ionic species.^{7–9}

Exploration in electrochemical systems of nanometer scale has been under intense investigations.^{10–13} In a highly confined cell geometry where the characteristic cell dimension is approaching or comparable to the typical Debye length, κ^{-1} , the surface charge

*Corresponding Author: Phone: 206 543 1767. Fax: 206 685 8665. zhang@chem.washington.edu.

ORCID: Jin Lu: 0000-0003-0905-0736; Bo Zhang: 0000-0002-1737-1241

Supporting Information: The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.anal-chem.6b02916.

Notes: The authors declare no competing financial interest.

on the cell walls or electrode may influence the distribution of charged redox species and their mass and electron transport processes. It has been previously reported that certain ions can be effectively accumulated or easily passed through a microscopic zone near to the charged electrode surface and other ions are repelled and excluded.^{14–16} This ion-enrichment/depletion effect has further been investigated for charge-sensitive biosensing, biomolecule separation, and seawater desalination applications.^{9,17,18} Additionally, the process of redox cycling can be used to amplify the faradaic signal when two electrodes are brought to close proximity or lithographically fabricated inside a microfluidic channel with nanometer spacing. Depending on the electrode spacing, a redox molecule can be oxidized and reduced more than 10^6 times resulting in the electrical detection of single molecules.^{19,20} Furthermore, the effects of flow rate, electric field, electrolyte concentration, and mass transport on the nanometer-sized electrochemical cell have also been well-studied.^{21–26}

In addition to their fundamental importance, miniaturized electrochemical systems also have practical significance in developing integrated electroanalytical devices for biomedical applications.^{27–30} The reduction of sensor size can achieve high sensitivity and spatial specificity, fast response, and high-throughput detection, as well as low material and energy cost. Recently, the use of modern lithography and microfabrication tools has facilitated the chip-based electrode and cell fabrication. In particular, it is becoming more feasible to fabricate the complex geometries and large arrays with miniaturized dimensions of the electrochemical systems.^{20,22,31,32}

Inspired by recent work of Lemay^{20,33} and White,^{22,34} we describe the preparation and characterization of an ultrathin-layer cell (UTLC) based on the formation of a nanometer-scale gap between a gold microelectrode and a glass substrate using a thin sacrificial layer of Cr. As shown in Scheme 1, the formation of a nanogap in a UTLC allows the precise placement of an active electrode above an insulating surface. The fabrication of the UTLC was accomplished by etching of a thin film of chromium (5–20 nm) evaporated between the glass and gold.

The thickness of the UTLC, d , was therefore defined by the Cr film and can be tuned as small as 5 nm. Compared with other thin-layer cells, our UTLCs were restrained by a gold microelectrode and a glass substrate without any risk of shorting between two parallel surfaces. Therefore, our UTLCs have a critical dimension in the range of 5–20 nm, which is significantly smaller than previously reported (i.e., 40–200 nm). This ultrasmall cell thickness enables us to explore electrostatic enrichment in extremely compact spaces with cyclic electrochemistry. The electrochemical property of the UTLC was thoroughly characterized with different redox molecules, supporting electrolytes, and scan rates. At low scan rates, the UTLC exhibited steady-state cyclic voltammetry (CV) response, which was dominated by radial type diffusion of redox from bulk to the edge of the gold. At higher scan rates, however, the electrode displayed transient CV response characteristic of a thin-layer cell behavior. Integration of the voltammetric peaks of faradaic current allowed us to quantify the number of redox molecules involved in the thin-layer response, which was largely contributed from the redox molecules present inside the UTLC.

Electrostatic force was found to play a critical role in UTLC's CV response. Electrostatic interaction between the redox and the UTLC's charged glass walls and the gold electrode determines the redox concentration in the nanogap. Interestingly, the use of chloride ions was found to have a strong effect on the CV response and enrichment factor of charged redox species, which is due to specific adsorption of Cl^- on gold. Cationic redox species, such like $\text{Ru}(\text{NH}_3)_6^{3+}$, showed an enhanced voltammetric response and could be dramatically enriched compared to neutral and anionic redox probes. An enrichment factor of ~ 100 was demonstrated for $\text{Ru}(\text{NH}_3)_6^{3+}$ in the presence of Cl^- . The use of numerical simulation provided additional insights toward a quantitative understanding of the electrochemical response of UTLCs. The simulation results confirmed that the coulometric integration was a good estimate of the redox molecules retained in the nanogap, and the errors existed for the different thin-layer geometries and experimental conditions were also discussed.

Experimental Section

Chemicals and Materials

Potassium chloride, potassium nitrate, sodium perchlorate, monosodium phosphate monohydrate, disodium phosphate heptahydrate, hexaammineruthenium(III) chloride, potassium ferricyanide, ferrocene methanol were all obtained from Sigma-Aldrich and used as received. Deionized water ($>18 \text{ M}\Omega\cdot\text{cm}$) was obtained through a Barnstead Nanopure water purification system and used for all aqueous solutions. Photoresist AZ 5214E and developer AZ 200 were purchased from AZ Corporation. Gold etchant TFA was purchased from the Transene Company.

UTLC Fabrication

Borosilicate glass slides (Thermos Scientific) were cleaned by rinsing thoroughly with deionized water and isopropyl alcohol and dried under a flow of pure nitrogen. Chromium (chrome-plated tungsten rods, 99.999%, Kurt J. Lesker) and gold (Au pellets, 99.999%, Kurt J. Lesker) were deposited on glass using an in-house thermal evaporator. The rate of metal deposition and the film thickness were monitored by a quartz crystal microbalance. A $2 \mu\text{m}$ layer of photoresist (AZ 5214E) was spin-coated on the Au film and patterned using a simple photomask to open a small circular window (typical $20\text{--}30 \mu\text{m}$ in diameter) in the polymer. The exposed Au microdisk was first etched with gold etchant, and the Cr layer was further etched by applying a $+1 \text{ V}$ dc potential in a 50 mM phosphate buffer at pH 7.4. The etching processes were monitored under the optical microscope (Figure 1b-d) and terminated immediately when the desired UTLC size was reached. A drop of electrolyte solution was kept on the top of the UTLC to maintain the nanogap geometry before and during the CV tests (Figure 1a, after step v). The fabrication process is highly repeatable with a success rate greater than 90%. The cell-cell variation comes mainly from the depth in Au/Cr etching, which can only be monitored and manually controlled to a certain extent under an optical microscope.

Cyclic Voltammetry

Cyclic voltammograms (CVs) were recorded using an in-house LabView program and a computer-controlled Dagan Chem-Clamp voltammeter. A PCI-5122 (National Instruments) data acquisition card and a BNC-2090 breakup box were used to interface the potentiostat and the PC and to digitize the current-voltage signal. A Ag/AgCl wire was used as a reference electrode (Figure 1a, after step v). For the slow scans (5 mV/s), the voltammograms showed the first full cycle. When the scan rates were higher than 1 V/s, the first multiple cycles were recorded (section 3 in the Supporting Information) and the averaged voltammograms were plotted for further analysis.

Numerical Simulation

The CV response was modeled using COMSOL Multiphysics 4.3a software on a 24-core PC workstation with 48 GB memory. Details of the model design and boundary conditions are presented in the Supporting Information.

Results and Discussion

Fabrication and Voltammetric Response of the UTLC

Figure 1a illustrates the general fabrication scheme of the UTLC. First, thin films of 20 nm Cr and 30 nm Au were evaporated on glass and a 2 μm film of photoresist was spin-coated on gold. Then, an ~ 20 μm Au disk was created by photolithography with a home-built UV photomask and the gold film was etched to expose the Cr surface. The gold etching may not always stop at the edge of the photoresist resulting in a slightly larger exposed Cr area compared to size of the polymer hole. This is shown in the optical micrographs displayed in Figure 1, parts b and c, in which the blue arrows mark the edge of the polymer hole and the yellow arrows show the gold edge. To create a UTLC, the Cr layer was further etched by applying a +1 V dc potential in a pH 7.4 phosphate buffer to form a nanogap between the Au and glass. The Cr edge is marked by the green arrow in Figure 1d, which is somewhat jagged after the etching. This is likely due to an uneven potential distribution along the Cr edges inside the extremely narrow space. The UTLC's critical dimension (or the gap height), d , was determined by the Cr thickness, which can be tuned in the evaporation process to as small as 5 nm. This dimension is much smaller than previously reported thin-layer cells^{22,33,35,36} and is important for the strong electrostatic enrichment effect observed in this work. As will be shown in the following sections, a large surface/volume ratio, which is the inverse of the cell thickness, would facilitate the high ion enrichment.

In addition to the use of optical microscopy, the etching processes were also monitored by cyclic voltammetry. Steady-state voltammetry provides an easy means to monitor the diffusive flux of redox to the surface of an ultramicroelectrode (UME), and the limiting current is determined by the mass-transfer resistance. Parts a–c of Figure 2 are CVs at 5 mV/s in solutions containing FcMeOH, $\text{Ru}(\text{NH}_3)_6^{3+}$, and $\text{Fe}(\text{CN})_6^{3-}$, respectively, all at 0.5 mM concentration, of the same UTLC prior to and after Au etching, and after Cr etching. The typical sigmoidal CVs were observed for all redox molecules on the slightly recessed Au disk electrode, and the CVs were in agreement with the simulation (Figure 2e, blue curve). Here, the diffusion coefficient of $0.8 \times 10^{-9} \text{ m}^2 \cdot \text{s}^{-1}$ was used in all simulation, which

is close to those of all three redox species: $D_{\text{FcMeOH}} = 0.78 \times 10^{-9} \text{ m}^2 \cdot \text{s}^{-1}$,³⁷ $D_{\text{Ru(NH}_3)_6^{3+}} = 0.84 \times 10^{-9} \text{ m}^2 \cdot \text{s}^{-1}$,³⁸ $D_{\text{Fe(CN)}_6^{3-}} = 0.73 \times 10^{-9} \text{ m}^2 \cdot \text{s}^{-1}$.³⁹ The radius of the exposed Cr surface was slightly increased from 9.0 to 12.9 μm due to over-etching of the gold (highlighted in red in Figure 1a steps iii, iv), and the simulation shows almost the same limiting current, i_{ss} (Figure 2e, red curve). The measured CVs follow the simulation results, with a slightly lower i_{ss} and potential shift likely due to possible oxidation of Cr during gold etching. After etching the Cr layer, a 20 nm nanogap was created and the active electrode area was further reduced to a 30 nm thick Au edge, the bottom of the ring-shaped Au surface recessed in the UTLC, and the 20 nm thick Cr edge.

The CVs show dramatically decreased currents, which are less than 50 pA for FcMeOH and Fe(CN)_6^{3-} and around 130 pA for $\text{Ru(NH}_3)_6^{3+}$ (insets in Figure 2a–c). The steady-state currents of FcMeOH and Fe(CN)_6^{3-} are in qualitative agreement with simulated result, after taking into account of the background voltammetry performed in the absence of redox probes (section 2 in the Supporting Information). $\text{Ru(NH}_3)_6^{3+}$ shows a higher steady-state current, which is attributed to an electrostatic attraction of $\text{Ru(NH}_3)_6^{3+}$ on the negative glass.

When the scan rate was increased to 5 V/s, the etched electrode displayed CVs with a pair of somewhat symmetrical redox peaks for $\text{Ru(NH}_3)_6^{3+}$ and FcMeOH, as shown in Figure 2d (please also see the raw CVs in Figure S2), which are characteristic thin-layer type CV behavior for an electrode of this size. A significant capacitive charging current was seen indicating larger electrode area/volume ratio in the nanogap. The peak current for FcMeOH was 40 pA, slightly greater than the simulated 16 pA current, (Figure 2f). Meanwhile, a 100-fold current enhancement was seen for $\text{Ru(NH}_3)_6^{3+}$ (1.7 nA). Because the peak current in a thin-layer cell is proportional to the number of redox molecules in the cell, one can quickly estimate an enrichment factor of 100 for $\text{Ru(NH}_3)_6^{3+}$ and ~ 2.5 for the neutral species FcMeOH.

The suppressing for negatively charged Fe(CN)_6^{3-} (not detectable) was also observed in the presence of KCl as the supporting electrolyte. It has been previously reported that Cl^- ions can be strongly adsorbed on Au,⁴⁰ and the glass substrate is also negatively charged at neutral pH.⁴¹ Both surfaces would then contribute to the formation of an electrostatic exclusion zone for negatively charged Fe(CN)_6^{3-} and an enriching zone for positive $\text{Ru(NH}_3)_6^{3+}$. The amount of the redox species (N_{exp}) in the UTLC can be more accurately determined from a coulometric integration of the peak current (section 7 in the Supporting Information). The N_{exp} value for $\text{Ru(NH}_3)_6^{3+}$ was 2.4×10^8 , which is 2 orders of magnitude higher than the number ($N_{\text{cal}} = 2.2 \times 10^6$) calculated from the UTLC volume, while N_{exp} of FcMeOH (2.4×10^6) is in fair agreement with N_{cal} value.

Electrolyte Dependence

The voltammetric response of UTLCs of the same three redox species was further studied in solutions containing different electrolytes. Parts a–c of Figure 3 show three sets of CVs of a 10 nm thick UTLC obtained at 20 V/s in solutions containing 0.1 M KCl, 0.1 M KNO_3 , and 0.1 M NaClO_4 , respectively. Three redox species were used in each case: 0.5 mM $\text{Ru(NH}_3)_6^{3+}$ (black), FcMeOH (red), and Fe(CN)_6^{3-} (blue). In KCl, similar to Figure 2d, $\text{Ru(NH}_3)_6^{3+}$ shows an enhanced peak current, and the weak peaks of both FcMeOH and

$\text{Fe}(\text{CN})_6^{3-}$ are not clearly resolvable with an increased background. When NO_3^- and ClO_4^- were used, however, the UTLC now exhibited resolvable CV peaks in FcMeOH and $\text{Fe}(\text{CN})_6^{3-}$ as shown in Figure 3, parts b and c. This is due to weaker adsorption of NO_3^- and ClO_4^- on gold⁴² leading to less negative charges on the gold surfaces. In order to further verify the proposed enrichment/exclusion zone, the CVs of a 15 μm Au disk ultramicroelectrode were also recorded. As shown in Figure S3, no significant differences of peak currents were observed in solutions containing different electrolytes. In another set of experiments, a 5 nm thick UTLC was tested. Under these conditions, similar voltammetric response was obtained for the 5 nm UTLC, as shown in Figure 3d–f.

We define an enrichment factor K as the ratio between the measured and calculated number of redox molecules ($N_{\text{exp}}/N_{\text{cal}}$), to quantitatively express how electrolyte ions affect the local concentration of a redox species in UTLC. For the 10 nm UTLC (Figure 3a–c), the values of N_{exp} , N_{cal} , and K are summarized in Table 1. $\text{Ru}(\text{NH}_3)_6^{3+}$ shows the highest enrichment factor ($K = 93$) in KCl solution, and it decreases to 67 and 37 in the presence of NO_3^- and ClO_4^- . The K value for $\text{Ru}(\text{NH}_3)_6^{3+}$ is always higher than that for FcMeOH and $\text{Fe}(\text{CN})_6^{3-}$ due to its positive charge and the negative glass surface. The observed K values for FcMeOH and $\text{Fe}(\text{CN})_6^{3-}$ were greater than 1 in the presence of NO_3^- and ClO_4^- . The larger K value may be attributed to several possible reasons including (1) a possible uncertainty when defining the baseline of a small peak current from the large background, which may lead to an excess coulometric integration; (2) the extra current/ coulomb contributions from the edge of the etched gold layer, which is discussed in more details in the following sections. We also used numerical simulation to further verify experimental observations and better understand how the CV and K value may be affected by the geometry of the UTLC and the scan rate (sections 5 and 6 of the Supporting Information).

Effect of KCl Concentration

We further studied UTLCs in 0.5 mM $\text{Ru}(\text{NH}_3)_6^{3+}$ with different KCl concentrations corresponding to $\gamma (= C_{\text{KCl}}/C_{\text{Ru}})$ increased from 0 to 2000, and the voltammetric results are presented in Figure 4a. No redox peak current was observed in the solution with low supporting electrolyte concentration ($\gamma = 0$ –2) suggesting that the 20 nm thick UTLC has a significant solution resistance and a large ohmic drop in the solution,⁴³ and no redox reaction can be observed on the electrode surface. The symmetrical redox peaks are only observed when γ is greater than 20, and the peak current increases from 0.09 to 0.24 nA (Figure 4c, open square). The capacitive current also increases as the supporting electrolyte concentration increases.

The CVs of the Au disk UME were also recorded in the same solution (Figure 4b). Interestingly, the peak current shows an opposite trend to the UTLC (Figure 4c). A highest peak current (0.76 nA) was observed when $\gamma = 0$. In the absence of excess supporting electrolyte, $\text{Ru}(\text{NH}_3)_6^{3+}$ is reduced to $\text{Ru}(\text{NH}_3)_6^{2+}$ at the electrode surface, resulting in the net negative charges. The presence of a high negative potential on the UME electrostatically attracts more $\text{Ru}(\text{NH}_3)_6^{3+}$ molecules to the electrode surface. The increased flux of reactant leads to a higher current. When $\gamma \gg 1$, this migration effect was effectively reduced by the use of excess supporting electrolyte, and a lower peak current is anticipated.

Numerical Simulation

In the simulation, we examine how each metal surface contributes to the overall CV response and how the K value may be affected by the scan rate, the Cr thickness, and the Cr layer etching depth (sections 5 and 6 of the Supporting Information), without considering any electrostatic effects. Figure 5 shows the simulated CVs with the UTLC geometry and the experimental conditions matching that used in Figure 3a. Details of the simulation models and parameters are given in section 1 of the Supporting Information. Figure 5a shows three ring-shaped surfaces, s1–s3, formed from the successive Au and Cr etchings. Boundary s1 is the 30 nm wide Au band perpendicular to the glass. Boundary s2 is the bottom surface of the exposed Au layer, and boundary s3 is the highly recessed 10 nm wide Cr. The total CV response was obtained from the redox current from all three boundaries.

The simulated results, including the total CV and the contributions of three boundaries, are presented in Figure 5b–d. The total CV response matches the experiments and changes from a steady-state sigmoidal shape to a peak-shaped thin-layer response, as the scan rate increases from 1 to 20 V/s. Boundary s1 is exposed to the bulk solution and is slightly recessed under the photoresist layer. The i - V response on s1 is sigmoidal and is mainly limited by redox diffusion from bulk to s1. A small volume of solution, ~ 10 fL, is confined in the nanogap, and there are 3.01×10^6 redox molecules enclosed inside. Therefore, boundary s2 shows two symmetrical CV peaks and contributes to the majority of the thin-layer response at higher scan rates. Boundary s3 is a highly recessed surface, and its contribution to the total current is negligible.

The amounts of redox species obtained by coulometric integration of the simulated thin-layer CVs (N_{sim} , N_{sim1} , N_{sim2} , N_{sim3} for total current, boundaries s1, s2, s3, respectively) are shown in Table 2. N_{sim2} is essentially stable from 1 to 20 V/s, and its value is very close to N_{cal} , suggesting that almost all the electroactive species trapped in the nanogap undergo a redox reaction at boundary s2. The contribution of boundary s1 (N_{sim1}) varies with scan rates and decreases from $0.73N_{\text{cal}}$ to $0.23N_{\text{cal}}$ as the scan rate increases from 1 to 20 V/s. Therefore, the coulometric integration of the experimental peak current (N_{exp}) is a high estimate for N_{cal} , resulting in a larger N value, and performing the CVs at a higher scan rate reduces this effect.

Conclusions

We have demonstrated a simple yet effective method to fabricate individual ultrathin-layer electrochemical cells with cell thickness as small as 5 nm and overall diameter of ~ 20 μm . Voltammetric response of such UTLCs depends on several factors including cell geometry, scan rate, supporting electrolyte, and charges on the redox species. At slow scan rates, it is characterized by a sigmoidal-shaped CV and is dominated by radial type diffusion of redox. At higher scan rates, e.g., >1 V/s, a peak-shaped CV response is obtained, which can be attributed to the redox behavior of molecules trapped inside the cell. A quick integration of the CV peaks allows one to quantify the amount of redox species present inside an ~ 10 fL cell volume. Our results show the cell walls and electrode surface can have a strong ion enrichment effect on charged redox species due to electrostatic interaction. The electrostatic interaction depends primarily on the negative surface charge of the cell walls but can also be

affected by surface adsorption on the gold electrode. We believe this simple UTLC design and electrostatic enrichment effect can be further utilized for future studies such as electrochemical sensing and charge-sensitive separations, e.g., desalination.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We gratefully acknowledge financial support from the Nation Science Foundation (CHE 1515897) and the National Institutes of Health (GM101133) and the University of Washington. We also thank Yunshan Fan for drawing the 3D view of the UTLC.

References

1. Wei C, Bard AJ, Feldberg SW. *Anal Chem.* 1997; 69:4627–4633.
2. White HS, Bund A. *Langmuir.* 2008; 24:2212–2218. [PubMed: 18225931]
3. Jin P, Mukaibo H, Horne LP, Bishop GW, Martin CR. *J Am Chem Soc.* 2010; 132:2118–2119. [PubMed: 20121145]
4. Kovarik ML, Zhou KM, Jacobson SC. *J Phys Chem B.* 2009; 113:15960–15966. [PubMed: 19908894]
5. Sa NY, Lan WJ, Shi WQ, Baker L. *ACS Nano.* 2013; 7:11272–11282. [PubMed: 24200344]
6. Guerrette JP, Zhang B. *J Am Chem Soc.* 2010; 132:17088–17091. [PubMed: 21073197]
7. Knust KN, Hlushkou D, Anand RK, Tallarek U, Crooks RM. *Angew Chem, Int Ed.* 2013; 52:8107–8110.
8. Kim SJ, Song YA, Han J. *Chem Soc Rev.* 2010; 39:912–922. [PubMed: 20179814]
9. Kim SJ, Ko SH, Kang KH, Han J. *Nat Nanotechnol.* 2010; 5:297–301. [PubMed: 20305644]
10. Li T, Hu W. *Nanoscale.* 2011; 3:166–176. [PubMed: 20967335]
11. Oja SM, Wood M, Zhang B. *Anal Chem.* 2013; 85:473–486. [PubMed: 23121243]
12. Oja SM, Fan Y, Armstrong CM, Defnet P, Zhang B. *Anal Chem.* 2016; 88:414–430. [PubMed: 26630546]
13. Fan Y, Han C, Zhang B. *Analyst.* 2016; 141:5474–5487. [PubMed: 27510555]
14. Pu QS, Yun JS, Temkin H, Liu SR. *Nano Lett.* 2004; 4:1099–1103.
15. Plecis A, Schoch RB, Renaud P. *Nano Lett.* 2005; 5:1147–1155. [PubMed: 15943459]
16. Wang YC, Stevens AL, Han JY. *Anal Chem.* 2005; 77:4293–4299. [PubMed: 16013838]
17. Fu JP, Schoch RB, Stevens AL, Tannenbaum SR, Han JY. *Nat Nanotechnol.* 2007; 2:121–128. [PubMed: 18654231]
18. Karnik R, Castelino K, Majumdar A. *Appl Phys Lett.* 2006; 88:123114.
19. Zevenbergen MA, Singh PS, Goluch ED, Wolfrum BL, Lemay SG. *Nano Lett.* 2011; 11:2881–2886. [PubMed: 21667924]
20. Kang S, Nieuwenhuis AF, Mathwig K, Mampallil D, Lemay SG. *ACS Nano.* 2013; 7:10931–10937. [PubMed: 24279688]
21. Mathwig K, Lemay SG. *Electrochim Acta.* 2013; 112:943–949.
22. Xiong J, Chen Q, Edwards MA, White HS. *ACS Nano.* 2015; 9:8520–8529. [PubMed: 26190513]
23. Ma C, Contento NM, Bohn PW. *J Am Chem Soc.* 2014; 136:7225–7228. [PubMed: 24805994]
24. Cao Z, Peng Y, Voth GA. *J Phys Chem B.* 2015; 119:7516–7521. [PubMed: 25556845]
25. Cooper JA, Compton RG. *Electroanalysis.* 1998; 10:141–155.
26. Branagan SP, Contento NM, Bohn PW. *J Am Chem Soc.* 2012; 134:8617–8624. [PubMed: 22506659]
27. Dawson K, Baudequin M, O'Riordan A. *Analyst.* 2011; 136:4507–4513. [PubMed: 21909570]

28. Jeong S, Park J, Pathania D, Castro CM, Weissleder R, Lee H. ACS Nano. 2016; 10:1802–1809. [PubMed: 26808216]
29. Bellin DL, Sakhtah H, Rosenstein JK, Levine PM, Thimot J, Emmett K, Dietrich LE, Shepard KL. Nat Commun. 2014; 5:3256. [PubMed: 24510163]
30. Liang X, Chou SY. Nano Lett. 2008; 8:1472–1476. [PubMed: 18416580]
31. Rassaei L, Singh PS, Lemay SG. Anal Chem. 2011; 83:3974–3980. [PubMed: 21510648]
32. Menke EJ, Thompson MA, Xiang C, Yang LC, Penner RM. Nat Mater. 2006; 5:914–919. [PubMed: 17057701]
33. Zevenbergen MA, Wolfrum BL, Goluch ED, Singh PS, Lemay SG. J Am Chem Soc. 2009; 131:11471–11477. [PubMed: 19722652]
34. Chen Q, McKelvey K, Edwards MA, White HS. J Phys Chem C. 2016; 120:17251–17260.
35. Hofmann B, Katelhon E, Schottdorf M, Offenhausser A, Wolfrum B. Lab Chip. 2011; 11:1054–1058. [PubMed: 21286648]
36. Lemay SG, Kang S, Mathwig K, Singh PS. Acc Chem. Res. 2013; 46:369–377.
37. Sun P, Mirkin MV. Anal Chem. 2006; 78:6526–6534. [PubMed: 16970330]
38. Wang Y, Limon-Petersen JG, Compton RG. J Electroanal Chem. 2011; 652:13–17.
39. Konopka S, McDuffie B. Anal Chem. 1970; 42:1741–1746.
40. Shi ZC, Lipkowski J. J Electroanal Chem. 1996; 403:225–239.
41. Kosmulski M, Matijevic E. Langmuir. 1992; 8:1060–1064.
42. Lipkowski J, Shi ZC, Chen AC, Pettinger B, Bilger C. Electrochim Acta. 1998; 43:2875–2888.
43. Goldberg IB, Bard AJ. J Electroanal Chem Interfacial Electrochem. 1972; 38:313–322.

Figure 1.

(a) Fabrication procedure of a UTLC: (i) thin-film thermal deposition of Cr and Au; (ii) spin coating of the photoresist; (iii) photolithography to create a circular window; (iv) Au etching in the Au etchant; (v) electrochemical etching of Cr by a +1 V dc potential in a phosphate buffer. The exposed electrode surfaces were highlighted in red. A drop of electrolyte solution was kept on the top of the UTLC to maintain the nanogap geometry before and during the CV tests. The schematic is not drawn to scale. (b–d) Optical micrographs of the same microelectrode after steps iii, iv, and v. Scale bars are all 10 μm in panels b–d. The arrows in panels b–d indicate the edges of the photoresist (blue), Au (yellow), and Cr (green).

Figure 2.

(a–c) CVs at 5 mV/s of the Au disk (blue), Cr disk (red), and UTLC (black) in FcMeOH, Ru(NH₃)₆³⁺ and Fe(CN)₆³⁻. Insets show the expanded CVs of the UTLC. (d) CVs at 5 V/s of the UTLC in FcMeOH, Ru(NH₃)₆³⁺, and Fe(CN)₆³⁻. (e and f) Simulated CVs of the Au disk (blue), Cr disk (red), and UTLC (black). Concentration of FcMeOH, Ru(NH₃)₆³⁺, Fe(CN)₆³⁻: 0.5 mM. Supporting electrolyte: 0.5 M KCl. Scan rate: 5 mV/s (solid line), 5 V/s (dashed line). Electrode: Au disk (1, blue), Cr disk (2, red), and UTLC (3, black), corresponding to Figure 1b–d. $R_{pr} = 9 \mu\text{m}$, $R_{Au} = 13 \mu\text{m}$, $R_{Cr} = 16.5 \mu\text{m}$, Cr layer thickness = 20 nm.

Figure 3.

CVs at 20 V/s of a 10 nm (a–c) and a 5 nm (d–f) UTLC in Ru(NH₃)₆³⁺ (black), 0.5 mM FcMeOH (red), and 0.5 mM Fe(CN)₆³⁻ (blue). Supporting electrolyte: 0.1 M KCl (a and d), 0.1 M KNO₃ (b and e), 0.1 M NaClO₄ (c and f). The UTLCs geometries: (a–c) $R_{pr} = 15.2 \mu\text{m}$, $R_{Au} = 16.8 \mu\text{m}$, $R_{Cr} = 24.5 \mu\text{m}$, Cr layer thickness = 10 nm; (d–f) $R_{pr} = 9.2 \mu\text{m}$, $R_{Au} = 11.6 \mu\text{m}$, $R_{Cr} = 13.8 \mu\text{m}$, Cr layer thickness = 5 nm.

Figure 4.

CVs of the UTLC (a) and a 15 μm Au disk UME (b) in 0.5 mM $\text{Ru}(\text{NH}_3)_6^{3+}$ with different KCl concentrations. γ ($C_{\text{KCl}}/C_{\text{Ru}}$): 0, 0.2, 2, 20, 100, 200, 1000, and 2000. Scan rate = 1 V/s. The UTLC geometry: $R_{\text{pr}} = 8.3 \mu\text{m}$, $R_{\text{Au}} = 10.9 \mu\text{m}$, $R_{\text{Cr}} = 15 \mu\text{m}$, Cr layer thickness = 20 nm. (c) The peak current of the UTLC (open square) and Au disk UME (open triangle) at different γ values. The solid triangle represents the peak current at $\gamma = 0$.

Figure 5.

(a) Scheme (not drawn to scale) of the UTLC used for the numerical simulation. The exposed electrode surfaces s1, s2, and s3 are highlighted in red. Simulated CVs (solid black) and the current contributions of the boundary s1 (dashed red), boundary s2 (dashed blue), boundary s3 (dash-dot cyan) at the scan rate of 20 (b), 5, and (c), 1 V/s (d). $R_{pr} = 15.2 \mu\text{m}$, $R_{Au} = 16.8 \mu\text{m}$, $R_{Cr} = 24.5 \mu\text{m}$, Cr layer thickness = 10 nm, [redox] = 0.5 mM.

Scheme 1.
Ultrathin-Layer Electrochemical Cell

Table 1
Number of the Redox Molecules (N_{exp}) and Enrichment Factor (K) Calculated from the 10 nm UTLC in Figure 3a–c

	no. molecules, N_{exp} ^a				enrichment factor (K)			
	KCl	KNO ₃	NaClO ₄		KCl	KNO ₃	NaClO ₄	
Ru(NH ₃) ₆ ^{3+-a}	2.80×10^8	2.01×10^8	1.11×10^8		93	67	37	
FcMeOH ^a	<i>c</i>	3.23×10^7	1.09×10^7		<i>c</i>	11	4	
Fe(CN) ₆ ^{3--a}	<i>c</i>	1.16×10^8	5.77×10^7		<i>c</i>	39	19	
N_{cal} ^b		3.01×10^6				1		

^aThe number of redox molecules determined by the peak integration in Figure 3a–c. The UTLC geometry: $R_{\text{pr}} = 15.2 \mu\text{m}$, $R_{\text{Au}} = 16.8 \mu\text{m}$, $R_{\text{Cr}} = 24.5 \mu\text{m}$, Cr layer thickness = 10 nm.

^bThe number of redox molecules in the UTLC, determined by the volume and the redox concentration.

^cToo small to be measured accurately.

Table 2
Number of Redox Molecules (N_{sim} , N_{sim1} , N_{sim2} , N_{sim3}) Integrated from the Simulated CV Responses at Different Scan Rates

	20 V/s		5 V/s		1 V/s	
	no. of molecules	ratio ($N_{\text{sim}}/N_{\text{cal}}$)	no. of molecules	ratio ($N_{\text{sim}}/N_{\text{cal}}$)	no. of molecules	ratio ($N_{\text{sim}}/N_{\text{cal}}$)
N_{sim}^a	3.55×10^6	1.18	3.87×10^6	1.28	5.20×10^6	1.73
N_{sim1}^a	6.94×10^5	0.23	0.97×10^6	0.32	2.21×10^6	0.73
N_{sim2}^a	2.85×10^6	0.95	2.90×10^6	0.96	3.00×10^6	0.99
N_{sim3}^a	2.32×10^3	0.00	2.32×10^3	0.00	2.23×10^3	0.00
N_{cal}^b			3.01×10^6			

^aThe number of redox molecules determined by the coulometric integration of the peak current from the simulated CVs in Figure 5. The UTL C geometry: $R_{\text{pr}} = 15.2 \mu\text{m}$, $R_{\text{Au}} = 16.8 \mu\text{m}$, $R_{\text{Cr}} = 24.5 \mu\text{m}$, Cr layer thickness = 10 nm.

^bThe number of redox molecules in the nanogap, calculated from the nanogap volume and the redox concentration.