Potential-Dependent Stochastic Amperometry of Multiferrocenylthiophenes in an Electrochemical Nanogap Transducer

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Supporting Information

ABSTRACT: In nanofluidic electrochemical sensors based on redox cycling, zeptomole quantities of analyte molecules can be detected as redox-active molecules travel diffusively between two electrodes separated by a nanoscale gap. These sensors are employed to study the properties of multiferroenylc compounds in nonpolar media, 2,3,4-triferrocenyli thiophene and 2,5-diferrocenyli thiophene, which display well-resolved electrochemically reversible one-electron transfer processes. Using stochastic analysis, we are able to determine, as a function of the oxidation states of a specific redox couple, the effective diffusion coefficient as well as the faradaic current generated per molecule, all in a straightforward experiment requiring only a mesoscopic amount of molecules in a femtoliter compartment. It was found that diffusive transport is reduced for higher oxidation states and that analytes yield very high currents per molecule of 15 fA.

1. INTRODUCTION

There is great interest in miniaturized electrochemical sensors for their ease of integration in lab-chip applications, highly sensitive detection, the requirement of only the smallest sample volumes, direct electrical signal transduction, and cost-effectiveness when standard microfabrication is employed.1−8 We previously reported on electrochemical nanogap sensors which allow sensitive analysis in femtoliter detection volumes.9 In these systems, electrochemically active analyte molecules shuttle by diffusion between two electrodes embedded in the roof and ceiling of a nanochannel (Figure 1). Thereby, the molecules undergo redox cycling, i.e., they are repeatedly oxidized and reduced at the opposing electrodes at kilohertz frequencies. In this way, each analyte contributes a current of several thousand electrons per second, resulting in a high intrinsic signal amplification (as compared to a single-electrode configuration in which a molecule reacts only once).

Nanogap sensors are currently rare in their ability to perform stochastic amperometric sensing.10 Only a small number of molecules are present in a very limited detection volume, even at high analyte concentrations. Therefore, the system can be described as a mesoscopic regime instead of a continuum: All molecules undergo a random Brownian walk and diffuse in and out of the nanofluidic channel and a coupled reservoir. This results in pronounced fluctuations of the molecular number density in the detection volume.11 These fluctuations are reflected in the recorded electrical current; they can directly be probed amperometrically. Molecular-level information is then gleaned from fluctuations in current—time traces. In particular, diffusion coefficients as well as specific dynamic adsorptivities were determined by analysis of the amplitude12 and frequency spectrum13,14 of current fluctuations.

To date, nanogap sensors have exclusively been employed for the detection of archetypical organometallic or metal—organic compounds such as ferrocenes15,16 or catechol derivatives;17,18 experiments were limited to conditions of a polar solvent (water or acetonitrile), a high concentration of fully dissociated background electrolyte, and analytes with typically only one possible electron-transfer process.
Electrochemical nanogap devices were fabricated by clean-room microfabrication on an oxidized silicon wafer substrate as described previously. They consist of a 5 μm wide, 10–20 μm long, and 70 nm high nanochannel in silicon oxide/silicon nitride; the roof and ceiling of the channel are Pt electrodes. The length and width of the nanochannel are defined by photolithography; the nanoscale height is defined by evaporation of a sacrificial Cr layer sandwiched between two Pt layers, which were also defined and fabricated by photolithography and electron-beam deposition, respectively. After these three metal deposition steps, the structure was buried in a Si3N4/SiO2 passivation layer by plasma-enhanced chemical vapor deposition. Access holes were subsequently plasma-etched into this layer to connect the nanodevice to a fluidic reservoir. Just before an experiment, the nanochannel was released by a selective wet-chemical etch of the sacrificial Cr layer (Cr etchant Selectipur, BASF). A micrograph and schematic view of a nanofluidic electrochemical device are shown in Figure 1.

2.2. Electrochemical Instrumentation and Experimentation. Using a home-built potentiostat setup, two sensitive operational amplifiers (Femto DDCPA-300) were separately connected to the top and bottom electrodes of a nanofluidic device. The amplifiers were LabView-controlled and used as current meters and voltage sources (Figure 1b). A micro-manipulator was used to position a reservoir in polydimethylsiloxane (PDMS) on top of the access holes. After the wet-etch of the chromium layer, the etchant was replaced by sulfuric acid (Sigma-Aldrich), prepared as a 0.5 M solution in Milli-Q water, and both Pt electrode surfaces were cleaned by repeatedly sweeping their potential between ~0.15 and 1.2 V versus a Ag/AgCl reference electrode (BASI inc.) placed in the reservoir.

The PDMS reservoir was then replaced by a reservoir machined in polytetrafluoro-ethylene and connected to the nanochannel via an O-ring (fluorinated propylene monomer, 0.5 mm inner diameter). The reservoir and nanochannel were filled with the analyte-containing dichloromethane solution, and a double-junction Ag/AgCl reference electrode (BASI inc.) was immersed into the reservoir. This reference electrode was constructed from a silver wire inserted into a Luggin capillary with a Vycor tip containing a solution of 0.01 M [AgNO3] and 0.1 M of the supporting electrolyte in acetonitrile. This Luggin capillary was inserted into a second Luggin capillary with Vycor tip filled with a 0.1 M supporting electrolyte solution in dichloromethane. The whole setup was shielded from interference in a Faraday cage.

3. RESULTS AND DISCUSSION

3.1. Cyclic Voltammetry. Cyclic voltammograms (CVs) of 2,5-diferoceynylthiophene and 2,3,4-triferrocenylthiophene (1 mM in 0.1 M [N′Bu4][B(C6F5)4] in dichloromethane) recorded at a Pt ultramicrodisk electrode (UME; BASI inc.) with a diameter of 10 μm are shown in Figure 2. In a stepwise oxidation, the analytes exhibit two (or three, respectively) well-resolved electrochemically reversible one-electron transfer processes with equal heights of the current steps. This is in good agreement with previous results.

By determining the magnitude of the steady-state currents, we estimate the diffusion coefficients, D, of the fully reduced species to be 1.08 × 10⁻⁹ m²/s for 2,5-Fc₂-C₄H₂S and 0.96 × 10⁻⁹ m²/s for 2,3,4-Fc₃-C₄HS. Diffusion coefficients (D) were estimated by $i = 4nFCDr$, where $i$ is the height of a current step,
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The number of exchanged electrons, \( n \), the analyte concentration, \( c \), and \( r \), the electrode radius, \( \frac{2}{3} \) of the number of exchanged electrons, \( F \), Faraday constant, \( e \) the charge in the number of exchanged electrons, \( \alpha \) the electrode radius. \( 0.1 \) M \([\text{N}^+\text{Bu}_4][\text{BF}_4]_{\text{i}}\)).

3.2. Cyclic Voltammetry in a Nanogap Device. Figure 3 shows voltammograms of both thiophene compounds recorded in a nanochannel device. The steps are not entirely as pronounced as for the measurements using the UME, which is most likely caused by the finite rate for heterogeneous electron transfer, as has been reported earlier for other couples.\(^\text{43} \) Nonetheless, all electron-transfer processes are clearly resolved. In these measurements, one electrode was kept constant at 0 V, while the other electrode was swept with respect to the reference electrode. The curves depict the oxidation current at one electrode and the reduction current at the other working electrode. This means that the molecules undergo redox cycling with one, two, or three electrons per molecule being exchanged during each process. While in the device, each molecule undergoes about 60 000 transfer processes per second. This leads to a considerable amplification of the current detected per molecule.

A difference in the measurements compared to the cyclic voltammetry at the UME is that the height of the current steps is reduced for an increasing magnitude of applied potentials or oxidation states, respectively, as indicated by the gray bars in Figure 3. This effect cannot be explained by a reduced diffusion coefficient of oxidized molecules or an effectively reduced diffusion due to dynamic adsorption; as such, a reduction is equilibrated by exchange (increase) in local concentration with the bulk reservoir.\(^\text{44} \)

We believe that the shape of the cyclic voltammograms recorded in the nanochannel is dictated by the finite rate for heterogeneous electron transfer, as has been reported earlier for other couples.\(^\text{45} \) We estimated the electrochemical rate constants \( k^0 \). As shown in the dashed lines in Figure 3, the forward sweeps of the CVs were fitted to the Butler–Volmer expression for thin layer cells,\(^\text{43} \) using a superposition of \( j \) one-electron reactions:

\[
\eta(E) = \sum_{j=1}^{n} \left[1 + \exp\left(-f(E-E_{\text{f}}^j)\right)\right] + \sum_{j=1}^{n} \frac{\eta_{\text{lim},j}}{e} \exp\left(-\alpha f(E-E_{\text{f}}^j)\right)
\]

Here \( \eta_{\text{lim},j} \) are limiting currents for the individual waves, \( E_{\text{f}}^j \) formal potentials, \( \alpha \) the transfer coefficient, \( z = 70 \) nm the nanochannel height, and \( f = F/RT \) \((F, \text{Faraday constant}; R, \text{gas constant}; T, \text{temperature})\). \( D_{\text{eff}} \) is the effective diffusion coefficient; diffusion is effectively slowed by dynamic analyte adsorption due to the high surface-to-volume ratio in the nanochannel of \( 3 \times 10^{-7} \text{m}^3 \), typically to \( D_{\text{eff}} = 0.2–0.5 \text{D} \) in previous experiments.\(^\text{10,15} \) The fitting parameters were \( \eta_{\text{lim},j} \) and the dimensionless rate constant \( D_{\text{eff}}/z^2k^0 \). The transfer coefficient was approximated by \( \alpha = 0.5 \).\(^\text{43} \) Formal potentials

![Figure 2. Cyclic voltammograms of 2,5-diferrocenyllithiophene (left) and 2,3,4-triferrocenyllithiophene (right) detected in dichloromethane solutions (1 mM) at a Pt ultramicroelectrode with a diameter of 10 \( \mu \)m].

![Figure 3. Cyclic voltammetry in the nanofluidic device. (a) 50 \( \mu \)M 2,5-Fc\(^+\)-C\(_7\)H\(_5\)S in a 20 \( \mu \)m long device. The top electrode is swept (red), while the bottom electrode is kept at 0 V (blue). The dashed line is a fit to the Butler–Volmer formalism, eq 1. Gray bars indicate limiting currents determined by the same fit. (b) 1 mM 2,3,4-Fc\(^+\)-C\(_7\)H\(_5\)S in a 10 \( \mu \)m long device. Here the bottom electrode is swept (red), while the top electrode is kept constant at 0 V (blue).]

![Figure 4. (a) Effective diffusion coefficient of 2,3,4-Fc\(^+\)-C\(_7\)H\(_5\)S determined by the magnitude of fluctuations \( \eta_{\text{diff}} \) in current–time traces. The “mean charge in e” corresponds to the applied potentials 0.5e \( \pm 0.25 \) V; 1e \( \pm 0–0.5 \) V; 1.5e (“0.3”) \( \pm 0–0.9 \) V; 1.5e (“1.2”) \( \pm 0.25–0.5 \) V; 2e \( \pm 0.25–0.9 \) V; 2.5e \( \pm 0.5–0.9 \) V. (b) Corresponding currents per molecule for 2,3,4-Fc\(^+\)-C\(_7\)H\(_5\)S. The gray lines indicate one-electron (lower line) and two-electron (upper line) transfer processes. (c) Effective diffusivities and currents per molecule for 2,5-Fc\(^+\)-C\(_7\)H\(_5\)S. Here the mean charge in e corresponds to the potentials 0.5e \( \pm 0–0.25 \) V; 1e \( \pm 0–0.55 \) V; 1.5e \( \pm 0.25–0.55 \) V.}
E° were determined as the inflection points of the CVs. Using the effective diffusion coefficients determined as shown below (see Figure 4), rate constants were extracted. They decrease considerably with a higher electron number from 1.4 to 0.50 cm/s for 2,5-Fc2-C6H4S and from 0.40 to 0.18 to 0.07 cm/s for the 2,3,4-Fc3-C6H4S compound for the first and second (and third) wave, respectively.

Moreover, electrical migration might have an adverse effect on transfer kinetics and analyte transport perpendicular to the electrodes; this effect was previously studied in nanogap geometries.\(^{45-47}\) [NBu4][B(C6F5)4] has a large ionic dissociation constant \(K_0 > 1000.\) Therefore, a thick electrical double layer of \(\lambda_{\text{D}} > 10\) nm (at actual electrolyte ion concentrations <0.1 mM) could lead to repulsion of charged analytes from charge electrodes and thereby to reduced limiting currents for higher oxidation states.

3.3. Stochastic Amperometry. We biased the electrodes a specific fixed potential, recorded current–time traces for both electrodes, and analyzed the mesoscopic number density fluctuations mirrored in the current fluctuations. In this detection scheme, the nanogap geometry offers the unique advantage that an arbitrary couple of oxidation states can be chosen for redox cycling between the two working electrodes. This means that also cycling between two positive oxidation states is possible, independent of the fully reduced bulk state.

Properties were analyzed by determining the magnitude of the fluctuations,\(^{12}\) i.e., the standard deviation \(i_{\text{std}}\) and comparing it to the magnitude of the Faradaic limiting current \(\langle i_{\text{lim}} \rangle\) for specific combination of electrode potentials. Here, \(i_{\text{std}}\) depends on the current generated per molecule, \(i_p\) and the mean number of analyte molecules \(\langle N \rangle\) present in the detection volume:

\[
i_{\text{std}} = i_p \langle N \rangle^{1/2}
\]

The current per molecule, \(i_p\) depends only on the nanochannel height, \(z\); the number of electrons transferred, \(n\); and the diffusivity, \(D_{\text{eff}}\) (and on the elementary charge, \(e\)):

\[
i_p = n e D_{\text{eff}} / z^2 \quad (n = 1, 2, 3)
\]

By using eqs 2 and 3 as well as the limiting current \(\langle i_{\text{lim}} \rangle = i_p \langle N \rangle\), \(D_{\text{eff}}\) and \(i_p\) can directly be determined, i.e., \(i_p = i_{\text{std}}^2 / \langle i_{\text{lim}} \rangle\).

The results of this fluctuation analysis are shown in Figure 4a. Here, the effective diffusion coefficient, \(D_{\text{eff}}\), is plotted as a function of applied potential represented by the nominal “mean charge in e". This means that for a value of 0.5, the analyte electroactive species \(2,3,4\)-Fc3-C6H4S cycles between the fully reduced state and a state with only one oxidized ferrocenyl group, potentials of 0 V and 250 mV are applied, and the molecules in the device have an average charge of about 0.5e. Our measurements show unequivocally that the effective diffusivity decreases with increasing mean charge or with the mean of the potential applied at the top and bottom electrodes. Figure 4b shows the current per molecule, \(i_p\), as determined by eq 3. This current increases with the number of molecules exchanged but decreases for higher oxidation numbers. In Figure 4c, the same trends of smaller currents and diffusion coefficients for an increased mean charge are depicted for the 2,5-Fc2-C6H4S analyte.

\(D_{\text{eff}}\) can also be extracted from a current–time trace by determining its power spectral density and determining the characteristic crossover frequency, \(f_c\).\(^{13}\) Both methods are compared in the Supporting Information, showing higher diffusivities for the latter methods but the same trend of reduced \(D_{\text{eff}}\) for higher potentials and charges.

The slightly faster diffusion of 2,5-Fc2-C6H4S compared to 2,3,4-Fc3-C6H4S is expected because of its smaller size. The observed reduced diffusivities at higher oxidation states are also not unexpected. For example, the diffusion coefficient of ferrocenedimethanol in aqueous solution is reduced by 20% in its oxidized state\(^{44}\) because of a changed solvation shell. In the case of the ferrocenyli thiophenes, a similarly strong effect is not expected because of the very large size of the electrolyte ions in a nonpolar solvent. Several origins or a combination of effects can lead to the observed change in \(D_{\text{eff}}\), namely, a change in bulk diffusivity, a change in dynamic adsorptivity, a slightly reduced limiting current due to slow kinetics, as well as a contribution by electrical migration. The increased \(D_{\text{eff}}\) for the spectroscopic determination compared to the current magnitude analysis (Figure S2 in the Supporting Information) hints at an anisotropic contribution by migration which is captured in \(i_{\text{std}}\) but not in \(i_p\) because only longitudinal diffusion contributes to the crossover frequency (shuttling in and out of the nanochannel), while fluctuations on all time scales (including diffusion across the channel) are captured in \(i_{\text{std}}\).

A change in electrostatic adsorption can be ruled out as a cause for the decreased effective diffusivity because more positively charged analytes would be repelled from more positively charged electrode surfaces, but a decrease in \(D_{\text{eff}}\) is observed for these conditions.

Ultimately, while clear trends are observed for \(D_{\text{eff}}\) and \(i_p\), so far it is not possible to distinguish the contribution of adsorption and changed diffusivities to these observations. While transport is hindered in the nanochannel, very large currents per molecule of up to 15 fA at room temperature were measured for high overpotentials (Figure 4b). This shows that compounds exhibiting multielectron transfer processes can have a potential application in single-molecule electrochemistry.

4. CONCLUSIONS

We introduced a new electrochemical method to determine the diffusive properties as a function of the oxidation state of complex electrochemically active molecules in a stochastic amperometric measurement. The experiments show that electrochemical nanogap sensors are suitable to work under conditions of a very volatile solvent, enabling a more complex analysis of novel compounds. For the investigated oligoferrocenyl thiophenes, our measurements show a decrease in effective diffusivity for an increase in applied potentials and oxidation state of the compounds. Moreover, highest currents per molecule of 15 fA per molecule were determined, which compares well to previous single-molecule experiments.\(^{16}\) For future experiments, we plan to investigate the origins of changes in effective diffusivity by determining the influence of electrical migration as well as to extend the method from identifying the properties of a redox couple to determining the diffusion of a specific oxidation state.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcc.6b07320.

Power spectral density analysis of current–time traces (PDF)
REFERENCES

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Supporting Information for:
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Electrochemical correlation spectroscopy

**Figure S1.** Power spectral densities (PSDs). a) PSDs for oxidation potentials of 0.9 V, 0.5 V and 0.25 V for 0.5 mM 2,3,4-Fc3-C4HS undergoing redox cycling in a 10 µm long device (the reduction potential is kept at 0 V). Gray lines are a fit to the theoretical model. Inset: raw current-time traces for potentials of 0.25 V and 0.9 V and the top and bottom electrode, respectively. b) Power spectra for 10 mM 2,5-Fc2-C4H2S in a 20 µm long device.
Exemplary power spectra extracted from current-time traces measured in a nanofluidic device are shown in Figure S1. Traces were recorded for a length of 60 s (100 s for 2,5-Fc₂⁻C₄H₂S) at a bandwidth of 150 Hz. Low-frequency drift was filtered, and spectra of three current-time traces were averaged for each depicted power spectrum. The PSDs $S(f)$ can be characterized by two characteristic properties: the magnitude of the plateau at lower frequencies, which for a given geometry and diffusion coefficient depends only on the analyte concentration, and the crossover frequency $f_0$, which indicates the transition from the plateau at lower frequencies to a $f^{3/2}$ decay at high frequencies [1]. The value of $f_0$ was extracted by a fit to $S(f) = S_0/(1 + (ff_0)^{3/2})$ and $D_{\text{eff}}$ evaluated using $f_0 = (D_{\text{eff}}/\pi)(3/L^2_a(L_a + 6L_e))^{2/3}$. Here $L_a$ is the top electrode length and $L_e = 2 \mu$m is the distance between the access holes and the top electrode. Figure S2 shows corresponding diffusivities for 2,5-Fc₂⁻C₄H₂S in comparison to $D_{\text{eff}}$ determined by evaluating the current fluctuation magnitude (as shown also in Fig. 4c).

![Figure S2. Comparison of $D_{\text{eff}}$ of 2,5-Fc₂⁻C₄H₂S calculated by determining $i_{\text{std}}$ and the crossover frequency $f_0$ of the power spectral density of a current-time trace.](image)

**Reference**